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OUR UNKNOWN PLANET: RECENT DISCOVERIES AND THE FUTURE. INTRODUCTION[1]

P. Mick Richardson[2]

Recent years have seen many new discoveries in the plant, animal, and other kingdoms. Can we estimate how many more organisms are out there to be discovered? An international group of experts has been invited to St. Louis to give their thoughts and predictions about this intriguing area of biology. The symposium will consist of an exciting set of presentations, ranging from flowering plants in the U.S., Australia, and the tropics, to freshwater fishes, mammals, and last, but not least in every sense, extremophiles and other bacteria.—Flyer advertising the 45th Annual Systematics Symposium.

In February 1998 I was in London, and purely by chance I was able to attend a talk at the Linnean Society on coelocanths, expertly delivered by Pete Forey. Later that year my wife and I were impressed by a coelocanth specimen in a delightful marine science museum in Tulear, a specimen caught locally off the southwest coast of Madagascar and thought to have been swept southwards from the populations around the Comoro Islands. However, coelocanths have recently been found in Indonesia, and one wonders if there are more of them around the world to be discovered. The finding of this second species of coelocanth was headline news to biologists, as was the discovery of the Wollemi Pine and several new large mammals, discussed in the following pages. However, the regular and continued reporting of new species usually attracts less attention. For example, one of my former students recently published five new species in the bromeliad genus *Cryptanthus* (Ramirez, 1998). To my mind, such a publication may actually be more important than the discoveries which make headline news because the new species of *Cryptanthus* are the result of full and detailed revisions of the genus based on field, herbarium, and laboratory studies. The readers can judge for themselves in the following papers that comprise the published proceedings of the symposium.

The 1998 Annual Systematics Symposium differed from the usual format in that there were ten speakers rather than the usual seven. This allowed for a greater diversity in composition of the program

[1] This and the eight articles that follow it are the proceedings of the 45th Annual Systematics Symposium of the Missouri Botanical Garden, *Our Unknown Planet: Recent Discoveries and the Future.* The symposium was held 9–10 October, 1998, at the Missouri Botanical Garden in St. Louis, Missouri, U.S.A.

The symposium was supported in part by the National Science Foundation under grant number DEB-9420140. I thank Peter Raven for helping to select a fine diversity of speakers, Kathy Hurlbert and her expert staff for wonderful help in organizing and administrating the symposium, Barbara Alongi for her fine illustration used for the cover of the symposium brochure, and the symposium speakers for being such a pleasant group of scientists.

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and allowed a wider variety of organisms to be covered in some detail. The morning session began with Michael Madigan's talk on prokaryotic organisms, a group that constitutes the bulk of evolutionary diversity on Earth and which is of increasing interest for use in biotechnology and related areas. Think about where systematics would be without the PCR methodology so necessary for current molecular systematics techniques. Literally, the book on molecular systematics of plants had to be rewritten within six years (Soltis et al., 1992, 1998). Microbes were followed by Richard Brusca's fascinating talk on arthropod diversification, and this made me think of eating deliciously tasty large crabs caught in the River Jurua in Acre, Brazil. Next was Ebbe Nielsen's discourse on insects, unfortunately not included in the published proceedings. Our current knowledge of freshwater fishes was the subject of John Lundberg and his colleagues. If South American fishes are so incompletely known, I wonder if the diversity of fishes we ate alongside the aforementioned crabs may have been species new to science. Fortunately, they were photographed before they went into the frying pan, leaving some clues at least to their existence. Last in the animal line of talks was John MacKinnon, who told us about new ungulates being discovered in Vietnam and his predictions about where future finds will likely be made. The morning session ended with some wonderful video footage and slides from Lynn Margulis, not published here, but see her books *Five Kingdoms: An Illustrated Guide to the Phyla of Life on Earth* (Margulis & Schwartz, 1998) and *Symbiotic Planet: A New Look at Evolution* (Margulis, 1998). While not everyone will agree with Margulis's concept of monophyly, there is no denying that she has added a very interesting viewpoint to overall discussions of biodiversity, and at the same time she makes an urgent appeal for all biologists and paleontologists to integrate their analyses and discuss criteria for establishment of higher taxa.

The plant talks came after lunch. Iain Prance informed the audience (and now the readers) that the number of angiosperms is currently underestimated, and he confidently predicted that there are in fact between 300,000 and 320,000. He used specific examples of the discovery of palms in Madagascar and other areas, as well as detailed studies of all genera in areas in Brazil and Brunei, to develop his case for an intensification of the rate of collection to confirm his predictions before it is too late. Barbara Briggs's talk on botanical discoveries in Australia contrasted the media attention given to the discovery of a new genus of conifers compared to the uncharismatic discovery of 61 new species in the Restionaceae and allied families. Finally, Barbara Ertter made the surprising announcement that the rate of discovery of new plants in the United States and Canada has been constant for the past century and shows no evidence of tapering off.

Mike Donoghue gave a very entertaining and stimulating after-dinner talk, emphasizing that the current age of discovery may be different from earlier ones, but it is both richer and more illuminating. It is the duty of all systematists to capture the imagination of other scientists and, even more importantly, the public at large.

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EXTREMOPHILIC BACTERIA AND MICROBIAL DIVERSITY[1]

Michael T. Madigan[2]

ABSTRACT

Many prokaryotic microorganisms inhabit "extreme environments"—habitats in which some chemical or physical variable(s) differs significantly from that found in habitats that support plant and animal life. Great strides have been made in recent years in the isolation and characterization of extremophilic prokaryotes, and many of them turn out to have fascinating metabolic properties and interesting evolutionary histories. Prokaryotes that grow at very high temperatures are perhaps the most dramatic in these regards, as all cellular components need to be made heat stable and their evolutionary position is that of the least evolved of all known life forms. As our knowledge of bacterial diversity improves, primarily from the introduction of molecular tools for assessing bacterial phylogeny and diversity and from new advances in isolation and laboratory culture, it is becoming clear that the bulk of evolutionary diversity on Earth does not reside in plants and animals, but instead in the invisible prokaryotic world. There is now great interest in mining the diverse genetic resources of Earth's smallest cells for use in biotechnology and related areas.

Key words: extremophilic bacteria, evolutionary history, microbial diversity, prokaryotes.

Since the days almost 100 years ago when Robert Koch and his associates isolated the first pure cultures of bacteria, microbiologists worldwide have been isolating laboratory cultures of literally thousands of different bacteria. These include, of course, most of the causative agents of infectious diseases, but more important from the standpoint of the web of life on Earth, many of the bacteria that carry out critical chemical reactions that form the "life support" system for plants and animals (Madigan et al., 2000). Despite the diversity of organisms that are already known, it is now clear that microbiologists have only seen the tip of iceberg; most microorganisms that exist in nature, in particular the bacteria, have not yet been obtained in laboratory culture! Indeed, with the help of new molecular tools microbiologists have explored a variety of microbial habitats and have detected not only new *species* of bacteria, but new *genera*, *families*, *orders*, and even *phyla* (Barns et al., 1994; Hugenholtz, et al., 1998). Imagine finding a new phylum of plants or animals today! The challenge for microbiologists now is to isolate these organisms, learn about their basic biology, and harness their vast genetic resources for the benefit of mankind.

A NATURAL PICTURE OF THE BACTERIAL WORLD

Great excitement has pervaded the field of microbial diversity in recent years because of the new-found ability of microbiologists to experimentally determine the evolutionary relationships of bacteria. This giant leap forward emerged from the tools of molecular biology, especially as regards the development of rapid gene sequencing methods and powerful algorithms for the comparative analysis of nucleic acid sequences. But for these advances to impact microbial evolution, a gene or genes that reflected the evolutionary history of an organism had to be identified. Such an evolutionary "Rosetta Stone" had long been sought, but not until the advent of comparative ribosomal RNA sequencing as a rapid and specific means for deducing bacterial phylogenies (Woese, 1987) did microbiologists have the tool they needed to classify bacteria in a *natural* fashion—the way botanists and zoologists had classified their subjects for over a century using primarily phenotypic characteristics such as bones or leaf arrangements as evolutionary guideposts.

Two key concepts have emerged from comparative molecular sequencing of ribosomal RNAs: (1) that cells evolved along *three* major lineages, the Bacteria, the Archaea, and the Eukarya, instead of just two, the prokaryotes and the eukaryotes (Fig. 1); and (2) that the evolutionary difference between a mouse and an elephant (or between *Chlorella* and *Trillium*, for the more botanically oriented) pales by comparison to the evolutionary distance between virtually any two common soil bacteria you might want to mention, like *Pseudomonas* and *Bacillus*.

The first of these conclusions, that prokaryotic life contains two major evolutionary lineages, is slowly but surely becoming mainstream thinking among microbiologists, and is even gaining support

[1] The research of M. T. Madigan is supported by National Science Foundation grant OPP 9809195.

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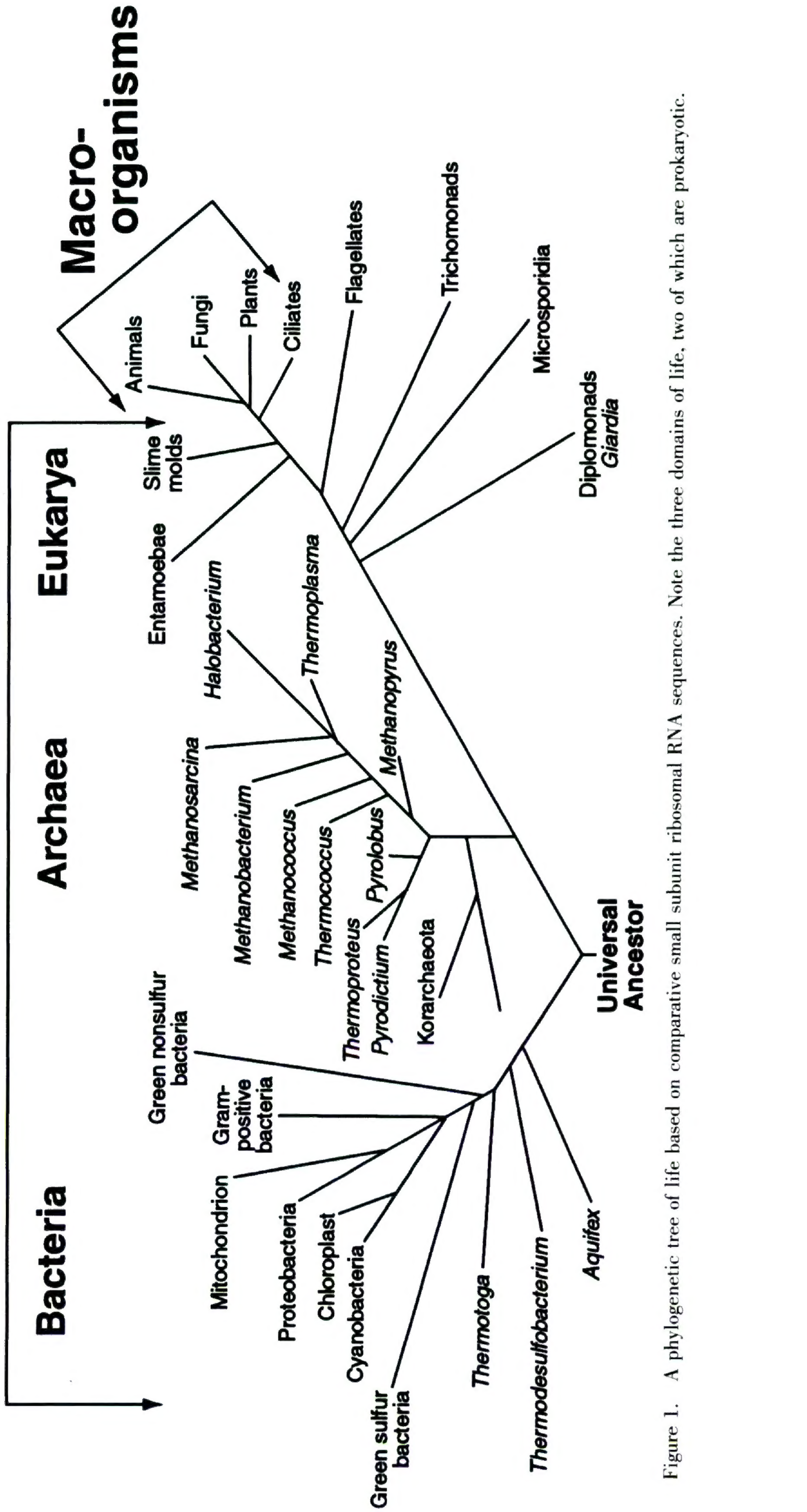

Figure 1. A phylogenetic tree of life based on comparative small subunit ribosomal RNA sequences. Note the three domains of life, two of which are prokaryotic.

from macrobiologists as evidenced by the inclusion of this concept in recent biology textbooks (Raven & Johnson, 1999; Raven et al., 1999). However, the second conclusion, that morphologically quite different plants or animals can be extremely closely related in a molecular evolutionary sense, has been for many a harder pill to swallow. If one steps back for a moment and considers that it is not the evolution of the mouse and the elephant, or the alga and the flowering plant, as intact entities, that molecular sequencing speaks to, but instead, the evolutionary history *of the cells that make them up,* it is easier to understand why the bulk of evolutionary change has occurred in the prokaryotic world; prokaryotes have existed for some 3.8 billion years, while the mouse and the elephant have only very recently evolved and diverged.

Prokaryotes ruled the Earth for at least 2 billion years before the modern (organelle-containing) eukaryotic cell appears in the fossil record. And metazoans (multi-celled plants and animals) have only existed for some half billion years or so. So by the time the stage was set for what botanists and zoologists consider the "evolutionary diversification of plants and animals," most of cellular evolution had already occurred. Diversification of the mouse and the elephant, for example, was simply a matter of arranging cells in different ways to yield what appears to the eye to be highly divergent organisms. But in terms of their evolutionary history, the mouse and the elephant are virtually identical organisms.

In contrast to higher organisms, prokaryotes have had more evolutionary time to show great genetic divergence. However, unlike metazoans, evolutionary change in prokaryotes is not manifest in morphological variation. For whatever reason(s), bacteria maintained a very small size and changed relatively little (compared with metazoans) in morphology through billions of years of evolutionary history. But that is not to say they did not evolve. Molecular sequencing tells us that they have indeed evolved but that the product of this evolutionary change is invisible—instead of big changes in size or shape, evolutionary change in the prokaryotes focused on metabolic diversity and the genetic capacities to explore and eventually colonize every conceivable environment on Earth, including extreme environments. Thus we must go to the *genes* of the prokaryotes to see their true phylogenetic diversification, and with advances in nucleic acid sequencing, this world is now beginning to open up (Madigan et al., 2000).

Using comparative ribosomal RNA sequencing microbiologists can now not only construct natural relationships of prokaryotes (Fig. 1), but can also use phylogenetic information to construct highly specific nucleic acid probes as a means of identifying and tracking specific microorganisms in the environment. A natural application of this technology has been to take these tools into various extreme environments and probe for the diversity of microbial life therein. The fallout from these studies, which historically followed by many years more classical enrichment and isolation approaches, has been an awareness that extreme environments are not a place for "hangers on," but instead are habitats that flourish with microbial life, especially prokaryotes (Barns et al., 1994; Horikoshi & Grant, 1998; Hugenholtz et al., 1998). The rest of this paper will try to introduce the reader to some of these organisms and their homes, and discuss what laboratory studies of these remarkable prokaryotes have revealed for our understanding of the physiochemical limits to life.

Extreme Environments and Extremophiles

Microbiological examination of extreme environments has revealed many new prokaryotes. By "extreme environment" here, it is meant an environment that humans would consider extreme or uninhabitable: extremes of heat or cold, pH, salinity, pressure, and even radiation. As previously mentioned, extreme environments are inhabited by diverse populations of microorganisms, most of which have evolved to live only in the presence of the extreme. These organisms are the "extremophiles" (Horikoshi & Grant, 1998; Madigan & Marrs, 1997; Madigan et al., 2000; Madigan & Oren, 1999). Several classes of extremophiles are recognized in microbiology, and laboratory cultures of representatives of each class are known (Table 1). Organisms in each class are denoted by a descriptive term, usually a word with Greek or Latin roots followed by the combining form "phile," Greek for "loving." Thus there are *thermophiles* and *hyperthermophiles* (organisms growing at high or very high temperatures, respectively), *psychrophiles* (organisms that grow best at low temperatures), *acidophiles* and *alkaliphiles* (organisms optimally adapted to acidic or basic pH values, respectively), *barophiles* (organisms that grow best under pressure), and *halophiles* (organisms that require NaCl for growth) (Table 1). Instead of trying to be inclusive here, as literally hundreds of different species could be included, the organisms listed in Table 1 are the current "record holders" in each of the extremophile categories. The column of most interest in Table 1 is the one labeled "optimum," for here

Table 1. Classes and examples of extremophiles[a].

Extreme	Descriptive term	Genus/species	Minimum	Optimum	Maximum
Temperature					
High	Hyperthermophile	*Pyrolobus fumarii*	90°C	106°C	113°C
Low	Psychrophile	*Polaromonas vacuolata*	0°C	4°C	12°C
pH					
Low	Acidophile	*Picrophilus oshimae*	−0.06	0.7 (60°C)[c]	4
High	Alkaliphile	*Natronobacterium gregoryi*	8.5	10 (20% NaCl)[d]	12
Pressure	Barophile	MT41 (Mariana Trench)[b]	500 atm	700 atm (4°C)	>1000 atm
Salt (NaCl)	Halophile	*Halobacterium salinarum*	15%	25%	32% (saturation)

[a] In each category the organism listed is the current "record holder" for requiring a particular extreme condition for growth.
[b] Strain MT41 does not yet have a formal genus and species name and is also a psychrophile.
[c] *P. oshimae* is also a thermophile, growing optimally at 60°C.
[d] *N. gregoryi* is also an extreme halophile, growing optimally at 20% NaCl.

it becomes clear that these organisms are not merely tolerating their lot, but that they actually do best in their punishing habitats; indeed most actually *require* their extreme condition(s) in order to reproduce at all.

Extremophiles are of interest to both basic and applied biology. In a basic sense, these organisms hold many interesting biological secrets, such as the biochemical limits to macromolecular stability and the genetic instructions for constructing macromolecules stable to one or another extreme (Madigan & Oren, 1999). But in an applied sense, these organisms have yielded an amazing array of enzymes capable of catalyzing specific biochemical reactions under extreme conditions (Adams & Kelly, 1995). Such enzymes have served as grist for industry in applications as diverse as laundry detergent additives (proteases, lipases) and the genetic identification of criminals (*Taq* DNA polymerase and its use in the polymerase chain reaction, PCR).

Another important realization that has emerged from the study of extremophiles is that some of these organisms form the cradle of life itself. Many extremophiles, in particular the hyperthermophiles, lie close to the "universal ancestor" of all extant life on Earth (Fig. 1). Thus, an understanding of the basic biology of these organisms is an opportunity for biologists to "look backward in time" so to speak, to a period of early life on Earth. This exciting realization has fueled much research on these organisms in order to understand the nature of primitive life forms, how the first cells "made a living" in Earth's early days, and how early organisms set the stage for the evolution of modern life forms.

Life at High Temperature

Although thermophilic bacteria (organisms with growth temperature optima between 45°C and 80°C) have been known for over 80 years, *hyperthermophilic* bacteria—organisms with optima above 80°C—have only been recognized more recently (Stetter, 1996). Following the pioneering work of Thomas Brock in the 1960s and 1970s (reviewed in Brock, 1978), Karl Stetter and co-workers at Regensburg (Germany) have proceeded to isolate over 30 genera (> 70 species) of hyperthermophiles. Brock was the first to demonstrate, often using simple but ingenious field experiments, that bacteria were present in boiling hot springs in Yellowstone National Park (Brock, 1978). By contrast, Stetter's group, whose focus has been on isolation and culture, has isolated many of the hyperthermophiles known today, including *Pyrolobus fumarii,* a remarkable prokaryote that can grow up to 113°C (Table 1, Fig. 2) (Blöchl et al., 1997).

Thermophilic microorganisms can be isolated from virtually any environment that receives intermittent heat, such as soil, compost, and the like. But hyperthermophiles thrive only in very hot and *constantly* hot environments, including hot springs, both terrestrial and undersea (hydrothermal vents), and active sea mounts, where volcanic lava is emitted directly onto the sea floor (Stetter, 1999). It is also strongly suspected, and some supportive evidence exists, that hyperthermophiles reside deep

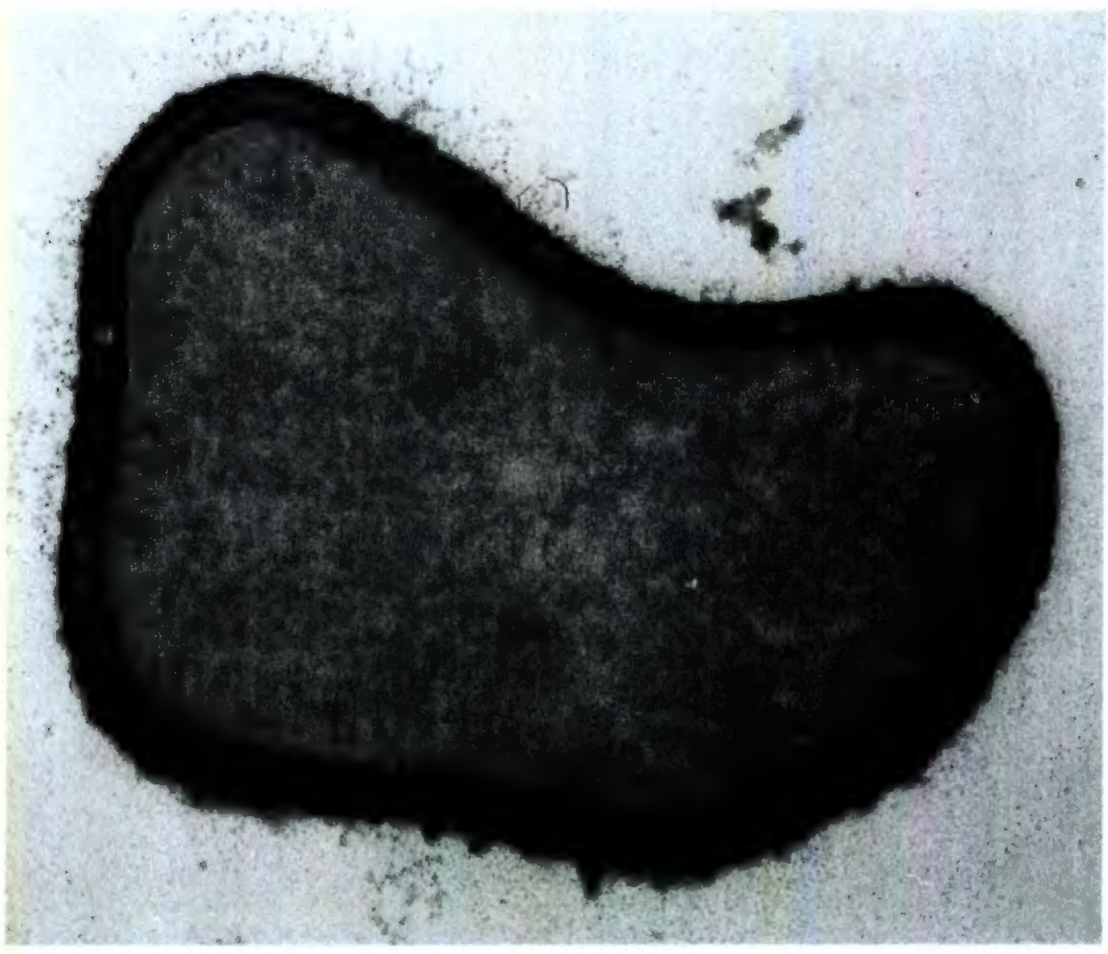

Figure 2. Transmission electron micrograph of a cell of *Pyrolobus fumarii*, the most thermophilic of all known living organisms. *Pyrolobus fumarii* grows optimally at 106°C and can grow at up to 113°C. Even higher temperatures are tolerated but do not support growth. Micrograph courtesy of Reinhard Rachel, Universität Regensburg.

within the earth, living a buried existence and relying on geothermal heat for their metabolic activities and reproduction (Stetter, 1999).

The most extreme of known hyperthermophiles, those with temperature optima *above* 100°C, have come from submarine hydrothermal vents (Stetter, 1996, 1999), and examples include *P. fumarii* (Blöchl et al., 1997, and Fig. 2) and the methanogen *Methanopyrus kandleri* (Kurr et al., 1991). Both of these amazing prokaryotes are members of the Archaea (Fig. 1) and are chemolithotrophs (organisms that use *inorganic* compounds as energy sources), using molecular hydrogen, H_2, as their electron donor (energy source), reducing either NO_3^- (*P. fumarii*) or CO_2 (*M. kandleri*) as electron acceptors to grow by anaerobic respiration (Madigan et al., 2000; Stetter, 1999). Besides *requiring* substantial heat for growth, these bacteria can survive temperatures substantially above their upper growth temperature limits, making a conventional autoclave regimen (15 min. at 121°C) insufficient for sterilizing cultures of either species!

Both *P. fumarii* and *M. kandleri* originated from hydrothermal vent chimneys (Blöchl et al., 1997; Stetter, 1999). These are precipitated iron mineral deposits that form as extremely hot water (up to 400°C) containing various minerals emerges from deep-sea hydrothermal vents (note that although this water is superheated, it does not boil because of the hydrostatic pressure of the water column, usually 2000–3000 m, that overlies these vents). Although the water that emerges is too hot for life, the chimneys, which are often only about 0.5 cm thick, show a temperature gradient from about 300°C inside to 2°C outside. Because prokaryotes are so small, microenvironments differing in temperature exist across the chimney wall leading to ideal habitats for various species of heat-loving bacteria.

Using nucleic acid probe technology several morphological types of bacteria have been detected in hydrothermal vent chimney walls (Harmsen et al., 1997), suggesting that these compact thermal gradients may contain many different microbial populations in addition to those already isolated. And for my botanical friends reading this paper, I would be remiss if I did not point out that *P. fumarii* and *M. kandleri* are good examples of primary producers totally divorced from sunlight, a capacity widespread in the microbial world. Besides growing at almost unbelievably high temperatures, *P. fumarii* and *M. kandleri* are also autotrophs, capable of growing in a simple anaerobic mineral salts medium supplied with CO_2 and H_2; neither sunlight nor a key product of photosynthesis, O_2, is required for either organism. Indeed, it has been hypothesized that long before the process of photosynthesis evolved, anaerobic H_2-based chemolithotrophy was the major means by which new organic material was synthesized on Earth (Madigan et al., 2000).

For an organism to grow at high temperatures, especially as high as those of the hyperthermophiles discussed here, *all* cellular components, including proteins, nucleic acids, and lipids, must be heat stable (Adams & Kelly, 1995; Ladenstein & Antranikian, 1998; Wiegel & Adams, 1998; van de Vossenberg et al., 1998a). The thermostability of enzymes from various hyperthermophiles, referred to as *extremozymes*, has been documented, and some have been found to remain active up to 140°C (Adams & Kelly, 1995). The structural features that dictate thermal stability in proteins are not well understood, but a small number of noncovalent features seem characteristic of thermostable proteins. These include a highly apolar core, which apparently makes the inside of the protein "sticky" and thus more resistant to unfolding, a small surface-to-volume ratio, which confers a compact form on the protein, a reduction in glycine content that tends to remove options for flexibility and thus introduce rigidity to the molecule, and extensive ionic bonding across the protein's surface that helps the compacted protein resist unfolding at high temperature (Ladenstein & Anthranikian, 1998). In addition to these intrinsic stability factors, special proteins called *chaperonins* are synthesized by hyperthermophiles. Chaperonins function to bind heat

denatured proteins and refold them into their active form. The *thermosome* is a type of chaperonin that is widespread among hyperthermophiles capable of growth above 100°C, like *P. fumarii* and *M. kandleri* (Stetter, 1999).

Several factors may combine to prevent DNA from melting in hyperthermophiles. However, the two most important features appear to be the enzyme reverse DNA gyrase, which catalyzes the *positive* supercoiling of closed circular DNA (by contrast, nonhyperthermophiles contain DNA gyrase, an enzyme that supercoils DNA in a *negative* twisted fashion), and various types of DNA binding proteins, including histone-like proteins (Madigan & Oren, 1999; Pereira & Reeve, 1998). For various physicochemical reasons, positively supercoiled DNA is more resistant to thermal denaturation than is negatively supercoiled DNA. And the fact that reverse gyrase seems to be the *only* protein thus far found universally among hyperthermophiles (regardless of their metabolic pattern) (Madigan & Oren, 1999) points to an important role for it in the heat stability of DNA.

Several hyperthermophiles contain DNA binding proteins that appear to play a role in maintaining DNA in a double-stranded form at high temperature. Some of these proteins are structurally related to the core histones of eukaryotic cells and function to wind and compact the DNA into nucleosome-like structures (Pereira & Reeve, 1998). Others have no structural relationship to histones but when bound to DNA alter its structure in such a way as to significantly raise its melting temperature (Madigan & Oren, 1999). It is likely that the combination of positive supercoiling of DNA along with proteins that prevent DNA melting is a major solution to the maintenance and integrity of DNA in hyperthermophiles.

Heat can also affect membrane stability. As all biologists know, in organisms living at moderate temperatures cell membranes are constructed along the typical "lipid bilayer" model: hydrophobic residues (fatty acids) inside oppose each other and retain an affinity for one another while hydrophilic residues (such as glycerol phosphate) lie at the surface of the environment and the cytoplasm, respectively, maintaining contact with the aqueous phase. If one applies sufficient heat to such a membrane architecture the two leaflets of the membrane will pull apart, leading to membrane damage and cytoplasmic leakage. To prevent this from occurring at very high temperatures, hyperthermophiles have evolved a novel membrane structure. Instead of forming a membrane as a lipid *bilayer*, as just discussed, some hyperthermophiles chemically bond the opposing hydrophobic residues from each layer of the membrane together (van de Vossenberg et al., 1998a). This forms a lipid *monolayer* instead of a bilayer, and prevents the membrane from melting at high temperature. Although the precise chemistry of lipid monolayer membranes can vary somewhat from species to species, they are common among hyperthermophiles and are likely an important evolutionary response to life at high temperature.

Life at Low Temperatures

How about life at the other end of the thermometer? Cold environments on Earth are actually much more common than hot ones. For example, the oceans, which make up over one half the Earth's surface, maintain an average temperature of about 2°C. And vast land masses are intermittently cold and in some cases permanently cold, or even frozen. However, cold temperatures are no barrier to microbial life, as various microorganisms flourish in cold environments, even in ice (Horikoshi & Grant, 1998; Madigan & Marrs, 1997). Many microorganisms have been isolated capable of growth at refrigerator temperatures (4–8°C). These are usually *psychrotolerant*, meaning that although they are capable of growth in the cold, they grow better at warmer temperatures, usually 25–35°C. True *psychrophiles*, defined as microorganisms that grow *best* at 15°C or lower, are usually only present in permanently cold environments like the Arctic, or in particular, the Antarctic (Horikoshi & Grant, 1998).

A variety of microorganisms including algae and diatoms have been found in Antarctic sea ice—ocean water that remains frozen for much of the year. Sea ice is the habitat for one well-characterized bacterium, *Polaromonas vacuolata*, the genus name indicating its affinity for cold temperatures (Irgens et al., 1996). *Polaromonas vacuolata* grows optimally at 4°C and finds temperatures above 12°C too warm for growth (Table 1). Other psychrophiles are known, but because some of them appear to be very sensitive to warming, great care must be taken in their isolation and culture to prevent killing them off at temperatures as low as room temperature.

An understanding of the biochemistry and molecular biology of psychrophilic bacteria is in a much earlier stage than that of the hyperthermophiles. From what is known about the biochemistry of psychrophiles, it appears that their proteins function optimally at low temperatures because they are constructed in such a way so as to maximize flexibility; this is essentially the opposite strategy from that of hyperthermophiles (see earlier). Moreover,

proteins from psychrophiles are typically more polar and less hydrophobic than proteins from hyperthermophiles, a fact that undoubtedly also assists in their relative flexibility.

Besides keeping their enzymes functional, psychrophiles have other biological problems to contend with, transport of nutrients across the membrane being chief among them. However, just as margarine, with its higher content of unsaturated fats, can stay softer than butter at cold temperatures, psychrophiles regulate the chemical composition of their membranes, including in particular the length and degree of unsaturation of fatty acids, to keep them sufficiently fluid to allow for transport processes, even at temperatures below freezing (Horikoshi & Grant, 1998). Applications of enzymes from psychrophiles include the cold food industry, where enzymes that work at refrigerator temperatures are sometimes desirable, as well as producers of cold-water laundry detergents (see more on this below).

Life in Battery Acid or Soda

Many extremophiles have evolved to grow best at extremes of pH: these are the *acidophiles* and the *alkaliphiles* (Horikoshi & Grant, 1998). Although extremely acidic or alkaline (below pH 3 or above pH 10) habitats are rare on earth, in such environments one can find a variety of microorganisms thriving in chemistry the equivalent of battery acid or soda-lime. Highly acidic environments can result naturally from geochemical activities, such as from the oxidation of SO_2 and H_2S produced in hydrothermal vents and hot springs, and from the metabolic activities of certain acidophiles themselves. For example, the iron sulfide-oxidizing bacterium *Thiobacillus ferrooxidans* can generate acid by oxidizing Fe^{2+} to Fe^{3+}, the latter of which precipitates out as $Fe(OH)_3$ ($Fe^{3+} + 3H_2O \rightarrow Fe(OH)_3 + 3H^+$), or by oxidizing HS^- to SO_4^{2-} ($HS^- + 2O_2 \rightarrow SO_4^{2-} + H^+$). *Thiobacillus ferrooxidans* is particularly active in surface coal mining operations where exposure to oxygen of pyrite (FeS_2) in the coal seam triggers acid production from the metabolic activities of this and related bacteria. Runoff from these habitats can often have a pH of less than 2, fueling conditions for further acidophile activity.

The most acidophilic of all bacteria known thus far is *Picrophilus oshimae*, whose pH optimum for growth is just 0.7 (Schleper et al., 1995) (Table 1). *Picrophilus oshimae* is also a thermophile (temperature optimum, 60°C) so this organism must be stable to both hot *and* acidic conditions. Cultures of *P. oshimae* were isolated from an extremely acidic (< pH 1) solfatara in Italy, and the organism has clearly evolved to require these highly acidic conditions for its very existence.

Interestingly, however, acid-loving extremophiles, even those as extreme as *P. oshimae*, cannot tolerate great acidity *inside* their cells, where it would destroy such important molecules as DNA. They thus survive by keeping the acid out. The internal pH of *P. oshimae* is about pH 5, and it is the cytoplasmic membrane of this organism that keeps protons from passively entering the cell. However, studies of the *P. oshimae* membrane have shown that it can only retain its integrity in acidic solutions; above an external pH of about 4 the *P. oshimae* membrane spontaneously disintegrates. Major unanswered questions concerning the metabolism of *P. oshimae* and other extreme acidophiles concern how they generate a proton motive force during respiration and related issues of bioenergetics involving membrane-mediated proton translocation (van de Vossenberg et al., 1998b).

Various acid-tolerant enzymes from acidophiles, primarily ones located on the cell surface or ones excreted from the cell into the acidic milieu, have been studied and potential industrial applications identified. These are primarily as animal-feed supplements where the enzymes function to break down inexpensive grains to more nutritionally beneficial forms directly in the animal's stomach. Such enzymes have been widely used in the poultry industry and have been shown to reduce feed costs and the time necessary to get birds to market.

Extreme alkaliphiles live in soils laden with soda (natron) or in soda lakes where the pH can rise to as high as 12. *Natronobacterium gregoryi* (Table 1), for example, was isolated from Lake Magadi, a soda lake located in the Rift Valley of Africa; *N. gregoryi* grows optimally at a pH of about 10 (Table 1) (Horikoshi & Grant, 1998). In the opposite scenario from the acidophiles, alkaliphiles have to contend with the problems associated with *high* pH. Above a pH of 8 or so, certain biomolecules, notably RNA, break down. Consequently, like acidophiles, alkaliphiles must maintain their cytoplasm nearer to neutrality than their environment. Nevertheless, any proteins found in the cell wall or in the membrane that make contact with the environment must be stable to high pH. Indeed, many such enzymes have been studied and a number have found industrial applications, especially in the laundry detergent industry. Detergents that are "enzyme enriched" contain proteases and lipases (enzymes that degrade proteins or fats, respectively, in clothing stains) that function at the high pH of soapy solutions (Horikoshi & Grant, 1998). In addition, alkali-

active enzymes from thermophiles and psychrophiles have been discovered and commercialized to better target detergent additives to hot water or cold water applications, respectively.

Besides keeping their cytoplasm near neutrality, alkaliphiles have other biological problems to contend with. For example, consider the problem of membrane-mediated bioenergetics—protons extruded to the external surface of the membrane enter a sea of hydroxyl ions. Nevertheless, biochemical studies of this problem have shown that a proton motive force is indeed formed by extreme alkaliphiles and drives some of the energy-requiring reactions in the cell, such as motility and transport. Sometimes in ATP synthesis, an ion gradient of Na^+, rather than H^+, drives this key bioenergetic process in extreme alkaliphiles (Horikoshi & Grant, 1998). This is probably not surprising when one considers that many (but not all) extreme alkaliphiles are also extreme halophiles (see below), requiring high salt as well as high pH for metabolism and reproduction.

Life in a Brine

Another remarkable group of extremophiles are the halophiles—organisms adapted to grow best in salty solutions (Oren, 1999; Ventosa et al., 1998). And for extreme halophiles like *Halobacterium*, a "salty solution" means anywhere from 25% NaCl up to saturation (32% NaCl) (Table 1). Halophilic microorganisms abound in hypersaline lakes such as the Dead Sea, the Great Salt Lake, and solar salt evaporation ponds. Such lakes are often colored red by the dense microbial communities of pigmented halophiles such as *Halobacterium* (Javor, 1989). Other habitats for halophilic microorganisms include highly salted foods, saline soils, and underground salt deposits. To date, a very large number of halophilic bacteria have been grown in culture including members of all domains of life, including the Eukarya (Kamekura, 1998). However, the archaeal halophiles as exemplified by *Halobacterium* species remain the most halophilic organisms known.

Halophiles are able to live in salty conditions by preventing dehydration of their cytoplasm. They do this by either producing large amounts of an internal organic solute or by concentrating an organic or inorganic solute from their environment (Horikoshi & Grant, 1998; Oren, 1999). The term "compatible solutes" is often used to describe organic osmolytes, of which there are several types, but not all halophiles employ such solutes (Madigan & Oren, 1999; Oren, 1999). For example, as its osmolyte, the archaeon *Halobacterium* (Table 1) concentrates large amounts of potassium (K^+, as KCl) from its environment. Dissolved KCl in the cytoplasm of *Halobacterium* cells is present at a concentration equal to or slightly above that of the dissolved NaCl outside, and in this way cells maintain the tendency for water to enter and thereby prevent dehydration. As would be expected from such a salty cytoplasm, enzymes that function inside of cells of *Halobacterium* have evolved to require this large dose of K^+ for catalytic activity. By contrast, membrane or cell wall-positioned proteins in *Halobacterium* require Na^+ and are typically stable only in the presence of high Na^+ (Madigan & Oren, 1999).

Extreme halophiles are sources of a variety of biomolecules that can function under salty conditions. Applications of salt-active enzymes include those that can break down viscous materials present in oil wells (oil is often found in geographic strata that contain salt) as well as enzymes that can carry out desirable transformations in highly salted foods. In addition, some halophiles that produce organic compatible solutes have been commercialized for the production of these solutes as skin care supplements (Madigan & Oren, 1999).

Other Extremophiles

Extremophilic microorganisms adapted to high pressure or which show no deleterious effects from exposure to high levels of radiation are also known. *Barophiles* are microorganisms that grow best under pressure greater than 1 atmosphere. Extreme barophiles are the most interesting in this regard as they actually *require* pressure, and in some cases, extreme pressure, for growth (Table 1). Strain MT41, for example, a bacterium isolated from marine sediments in the Mariana Trench near the Philippines (a depth of greater than 10,000 m), requires *at least* 500 atmospheres of pressure in order to grow and grows optimally at 700 atmospheres (and at a temperature of 4°C because strain MT41 is also a psychrophile). Because laboratory culture of extreme barophiles is rather difficult, comparatively little is known about their important biomolecules. However, although probably all macromolecules in extreme barophiles need to be biochemically tailored to high pressure to some extent, experiments with moderately barophilic bacteria, some of which can be grown without pressure, have pointed to nutrient transport proteins in the cytoplasmic membrane as key cell components requiring structural modifications in order to function at high pressure (Horikoshi & Grant, 1998).

The bacterium *Deinococcus radiodurans* is an amazingly radiation-resistant microorganism (Murray, 1992). This remarkable organism can survive 30,000 Grays of ionizing radiation, sufficient to literally shatter its chromosome into hundreds of pieces (by contrast, a human can be killed by exposure to as little as 5 Grays). A powerful DNA repair machinery exists in cells of *D. radiodurans* that is able to piece the shattered chromosome back together and yield viable cells. Because of its remarkable radiation resistance, *Deinococcus* has been proposed as a cleanup agent for the bioremediation of toxic materials in contaminated soils that are also radioactive from the leakage of radioactive materials; these conditions exist primarily at nuclear weapons production sites.

Extremophiles in the Evolution of Life

A focus of research on extremophiles has centered on the hyperthermophiles. As discussed earlier, there is good reason to believe that at least some hyperthermophiles have evolved relatively little from their ancestors present on earth over 3.5 billion years ago (Figs. 1, 2). If true, an understanding of the biology of hyperthermophiles may yield a glimpse of what life was like eons ago. In this connection the genomes of several hyperthermophiles have been sequenced (Madigan & Oren, 1999), and the large number of genes they contain that lack counterparts in other organisms suggests that their biological secrets have at this point only been partially revealed. As if living in boiling water isn't enough, just imagine what other tricks hyperthermophiles might be able to perform!

As previously mentioned, the excitement in microbial diversity these days comes from the fact that the evolutionary history of the prokaryotes can now be experimentally determined. Microbiologists no longer have to propose bacterial phylogenies based on speculation or "educated guesses" of what type of microbe likely preceded another; the phylogenies themselves are etched in the sequences of molecules, and all one has to do is read them. Moreover, the application of molecular phylogenetic methods to natural environments (Barns et al., 1994; Hugenholtz et al., 1998) has given us the exciting news that the diversity of the microbial world is enormous—indeed it is beyond our wildest expectations. Thus, in the final analysis bacterial diversity will likely dwarf that of all of the rest of biology, perhaps by several orders of magnitude. But only continued and expanded research into the diversity of microbial life in all environments, extreme and otherwise, will yield the data needed to confirm this.

It may indeed be humbling to many biologists to think that prokaryotes dominate living diversity. But within the rich genetic resources of the prokaryotes undoubtedly lies more benefit for humankind than we will extract from any other group of organisms. Antibiotics, fermentation, and biotechnology are only the beginning. The best is yet to come.

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UNRAVELING THE HISTORY OF ARTHROPOD BIODIVERSIFICATION[1]

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Abstract

Current views of arthropod phylogeny are assessed in light of recent research in morphological and molecular phylogenetics, developmental biology, neurobiology, and paleontology. Recent fossil discoveries and molecular clock data inform us that arthropod diversification began in the Precambrian, and suggest that by the Cambrian the arthropods were already the most speciose metazoan phylum on earth. The combination of metamerism and jointed appendages (with intrinsic musculature), and the evolutionary potential of homeotic genes, has profoundly affected arthropod evolution and created many morphological homoplasies. Evidence strongly favors a monophyletic Arthropoda. Accumulating evidence supports a hypothesis that insects and modern crustaceans comprise a phylogenetic sister group, and that they, and perhaps also trilobites, chelicerates, and myriapods, all could have evolved out of an ancient crustacean stem line. Two implications of this hypothesis are that Crustacea comprise a paraphyletic taxon and insects may be viewed as "flying crustaceans."

Key words: Arthropoda, arthropod evolution, Crustacea, insects, Metazoa.

Preface. The Challenge of Unraveling Metazoan Phylogeny

Despite great progress made in zoology during the 20th century, there remain many fundamental, unanswered questions concerning metazoan evolutionary history. The origins and relationships of many animal phyla remain unclear or in dispute. In large part, this stems from the challenge of recovering unambiguous phylogenetic signals from ancient lineages. Recent molecular and paleontological studies suggest that major splits among the Metazoa occurred in the Precambrian, some perhaps nearly a billion years ago (Wray et al., 1996; Ayala et al., 1998; Seilacher et al., 1998; Li et al., 1998). In part, it may also be because the field of comparative morphology has lost popularity (and employment opportunities). And finally, the emerging field of molecular phylogenetics is still so new that every year sees improvements in the data analyzed and the phylogenetic inference methods used. For example, prior to 1997 most molecular analyses were based on small numbers of taxa and short sequences of a single gene, usually the inherently problematic 18S rDNA gene. Recently, however, new (and larger) molecular data sets have been developed based on other conserved nuclear genes, mitochondrial gene order, and gene duplication data. Because it is unlikely that a single gene will recover the full phylogeny of Metazoa, the future will no doubt see analyses of multiple gene sets.

Emerging molecular studies have corroborated many, and challenged some, paradigms of metazoan phylogeny. For example, whereas some molecular studies have supported the long-held close relationship between annelids and arthropods (Wheeler et al., 1993), recent studies have not done so (Lake, 1990; Halanych et al., 1995; Eernisse, 1997; Aguinaldo et al., 1997). Furthermore, the discovery of new animal phyla, and thus new fundamental body plans, continues to occur. The first edition of Linnaeus's (1735) *Systema Naturae* listed 14 groups that we now recognize as distinct animal phyla. Today, we recognize 34 animal phyla. Three former phyla have recently been sunk: Pentastomids are now placed within the Arthropoda (allied with the Maxillopoda), and vestimentiferans and pogonophorans are now regarded as annelids (probably highly modified polychaetes) (McHugh, 1997; Brusca & Brusca, in press).

Most of the large-bodied animal groups were discovered by the end of the 19th century. We are now on a track of discovery of microscopic metazoa, and three new animal phyla have been discovered just since 1956: Gnathostomulida (1956), Loricifera (1983), and Cycliophora (1995). There is a correlation between the discovery of new animal phyla and their body sizes: phyla described in the period of 1901–1920 have maximum body lengths of 3–10 mm; phyla described in the period of 1941–1960 have maximum body lengths of just 1 mm;

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phyla described in the 1980s and 1990s have maximum body lengths of less than 0.5 mm. Most of the small-bodied phyla are meiofaunal, although cycliophorans live as commensals on the mouth appendages of various marine crustaceans (Funch & Kristensen, 1997). The discovery of these minute animals presents challenges to those of us interested in animal phylogeny. They are so small that a great deal of their anatomy is reduced or otherwise altered. We know almost nothing about their developmental biology, and they are so rare that molecular biologists have not yet extracted gene sequences from them. I predict that the discovery of new microscopic phyla will continue for another half-century.

The challenges of unraveling animal phylogeny are not unique to molecular biology, small animals, or new phyla. Biology has a long history of skirmishing over phylogenetic issues at all levels. The evolutionary history of the Arthropoda has been one of the most challenging issues biologists struggled with throughout the 20th century. What follows is an update (as of mid 1998) on what we know about arthropod evolutionary history.

Arthropod Evolution: Background

There are five clearly distinguished groups of arthropods: trilobites (extinct since the end of the Paleozoic; ~ 4000 described species); Cheliceriformes (horseshoe crabs, eurypterids, arachnids, pycnogonids; ~ 75,000 described living species); crustaceans (crabs, shrimp, isopods, and their kin; ~ 50,000 described living species); hexapods (insects and their kin; 878,000 to 1.5 million described living species); and myriapods (centipedes, millipedes, and their kin; ~ 14,000 described living species). And, there are two close allies of the arthropods, tardigrades (water bears) and onychophorans (*Peripatus* and their kin). The close relationship between the Tardigrada and the Arthropoda has never been seriously questioned (Brusca & Brusca, 1990), and recent molecular work continues to support a sister-group relationship between these two phyla (Garey et al., 1996). There are now 1.02 to 1.64 million described arthropods, known from virtually all environments on earth. Estimates of undescribed arthropod species range from 3 to 100 million. The arthropods (Table 1) comprise about 85% of all described metazoan species.

The arthropods also encompass an unparalleled range of structural and taxonomic diversity, have a rich fossil record, and have become favored animals of evolutionary developmental biology. Arthropods were among the earliest animals to evolve.

Table 1. Fossil record of major arthropod groups.

Tardigrades: Middle Cambrian to present
Onychophora: Middle Cambrian to present
Trilobita: Early Cambrian to Permian
Xiphosura: Early Ordovician/Silurian to present
Eurypterida: Early Ordovician through mid-Permian
Arachnida: Upper Silurian to present
Pycnogonida: Devonian to present
Crustacea: Early Cambrian (or Vendian) to present
Hexapoda: Lower Devonian to present
Myriapoda: Upper Silurian to present

Recent work (Waggoner, 1996) suggests that even the Ediacaran (Vendian) fauna, of the latest Precambrian, included early arthropod taxa, perhaps true Crustacea.

Ever since Darwin, biologists have asked the question, "How has the incredibly successful diversification of arthropods come about?" Why are there so many arthropods? Is there something "special" about these animals? What is the phylogenetic history of the Arthropoda? Specifically, are the arthropods monophyletic and what are the relationships of the major arthropod groups to one another? There have been four great challenges to biologists in answering these questions. (1) Until the last decade of the 20th century, there had been a lack of hypotheses on arthropod evolution based on principles of explicit phylogenetic inference. (2) We have a very incomplete understanding of arthropod development, though this is improving quickly with the advent of molecular developmental biology. (3) There has been a paucity of comprehensive studies based on fossils from the earliest ages of arthropod evolution (late Precambrian and early Paleozoic). (4) It is apparent that high levels of homoplasy exist among the arthropods. In just the past 10 years, major discoveries have begun to address each of these challenges, as discussed below.

Work by the great comparative biologist Robert Snodgrass in the 1930s established a benchmark in arthropod biodiversity research. Table 2 shows a classification of the arthropods at that time, and it is this classification that one still finds in most modern biology textbooks. The Snodgrass classification embraces three important hypotheses:

(1) Arthropods comprise a monophyletic taxon.
(2) Myriapods and hexapods form a sister group, a taxon called Atelocerata (= Tracheata, or Uniramia of some authors). The Atelocerata have been united by several seemingly powerful attributes:

Table 2. Classification of the arthropods and their allies sensu Snodgrass (1938).

Phylum Arthropoda
- Subphylum Trilobita
- Subphylum Chelicerata
  - Class Merostomata
    - Subclass Xiphosura. Horseshoe crabs
    - Subclass Eurypterida. Eurypterids; extinct Paleozoic arthropods
  - Class Arachnida. Land spiders, mites, etc.
  - Class Pycnogonida. Sea spiders
- Subphylum Mandibulata
  - Class Crustacea. Crabs, shrimps, isopods, etc.
  - Class Tracheata (= Atelocerata)
    - Subclass Hexapoda
      - Superorder Protura. Proturans
      - Superorder Insecta. Insects
    - Subclass Myriapoda
      - Superorder Chilopoda. Centipedes
      - Superorder Diplopoda. Millipedes
      - Superorder Symphyla. Symphylans
      - Superorder Pauropoda. Pauropodans

(a) A tracheal respiratory system.
(b) Uniramous legs.
(c) Use of Malpighian tubules for excretion.
(d) Loss of the second head appendages—the second antennae (as the name Atelocerata implies). Vestiges of the anlagen of this appendage can be seen during the embryogeny of some insects (e.g., Sharov, 1953; Brukmoser, 1965).

(3) The Crustacea and the Tracheata form a sister group, the Mandibulata—a name that Snodgrass himself coined.

For a brief period of time in the mid-century the concept of a polyphyletic Arthropoda, championed mainly by S. Manton and D. Anderson, enjoyed some popularity (Manton, 1973, 1977; Manton & Anderson, 1979; Anderson, 1979), and Anderson (1996) still maintains this view. The Mantonian view of arthropods placed the myriapods, hexapods, and onychophorans in a separate lineage (Manton's phylum "Uniramia") with an origin apart from the rest of the arthropods. However, this idea, based on flawed phylogenetic argumentation and an inadequate embryological foundation, did not long survive the rigors of scientific testing and modern methods of phylogenetic inference (see below). In addition to phylogenetic analyses, studies of Permian diaphanopteroid insects (Kukalová-Peck, 1991a, b, 1992; Kukalová-Peck & Brauckmann, 1990) have shown that early pterygotes probably possessed polyramous appendages, further undermining the Manton-Anderson Uniramia hypothesis. Additional support for arthropod monophyly has come from studies of compound eyes using a monoclonal antibody raised against a specific glycoprotein (3G6), to crystalline cones, eucones, and other elements in a variety of insect and crustacean retinas (Edwards & Meyer, 1990).

It was not until the late 1980s that Snodgrass's long-standing view of arthropod relationships began to be seriously questioned with: (1) the appearance of explicit morphological and molecular phylogenetic analyses, (2) the discovery of the amazing potential of homeobox genes in arthropod development and evolution, (3) the emergence of molecular-based evolutionary developmental biology, and (4) the discovery of exquisite new Cambrian preservations from Sweden, China, and elsewhere.

Morphological Phylogenetic Studies of Arthropods

Morphological phylogenetic studies of the arthropods are summarized in Table 3. Overall, these analyses suggest three important conclusions:

(1) The arthropods are a monophyletic taxon.
(2) The relationship of the Crustacea to the insects and myriapods is ambiguous; that is, Snodgrass's Mandibulata is a taxon of questionable validity.
(3) The monophyly of the Atelocerata (insects + myriapods) is also questionable.

Waggoner (1996) included in his analysis a number of arthropod-like fossils belonging to the "Vendian fauna," from the latest Precambrian (= Ediacara Period) that had generally been regarded as

Table 3. Morphological views of monophyly within the arthropods.

Year	Author(s)	Arthropods monophyletic	Mandibulates monophyletic	Tracheata monophyletic
1990	Brusca & Brusca	Yes	Yes	n.a.
1991	Schram	Yes	No	n.a.
1992	Eernisse et al.	Yes	n.a.	n.a.
1993	Backeljau et al.	Yes	n.a.	n.a.
1993	Wheeler et al.	Yes	Yes	Yes
1994	Wills et al.	Yes	Yes	Yes
1995	Wills et al.	Yes	No	No
1996	Nielsen et al.	Yes	Yes	Yes
1996	Waggoner	Yes	No	No
1997	Emerson & Schram	Yes	No	No
1998	Strausfeld	Yes	No	No

"problematica." He also included 21 Cambrian arthropods, and various modern taxa. He concluded that: (a) the Arthropoda are monophyletic, (b) the Ediacaran arthropod-like fossils are, in fact, true arthropods, and (c) the anomalocarids (and their kin) fall out very close to the base, and are probably the most primitive known arthropods. Anomalocarids were giant predatory arthropods (arguably, true Crustacea) that reached a meter in length. They are known from both the Precambrian and the Cambrian, and they were probably the largest predators of that time (Briggs, 1994; Chen et al., 1994).

The most recent phylogenetic analysis of arthropods was based on anatomical features of the central nervous system (Strausfeld, 1998). Strausfeld used 100 conserved neural characters in the brains of a variety of segmented invertebrates to reconstruct phylogenetic relationships among the arthropods. His analysis suggested that insects and crustaceans comprise a sister group, that the myriapods are a polyphyletic group (i.e., chilopods and diplopods are not sister taxa), and that pycnogonids are true chelicerates. The most important neuronal synapomorphies of Crustacea–Insecta are elements of the optic lobes and mid-brain, particularly features of the midline neuropils and neuropils associated with the compound eyes. This analysis corroborated earlier neurological descriptive work by Strausfeld et al. (1995), which also concluded that insects are closer to crustaceans than to any other arthropod group.

All arthropod central nervous systems use the same fundamental embryonic plan of construction (Whitington et al., 1993; Thomas et al., 1984; Strausfeld, 1998). However, a fundamental distinction between the early embryonic development of the myriapod nervous system and that of insects + crustaceans was recognized some time ago. Whitington et al. (1991) found that in insects and crustaceans longitudinal connectives are pioneered by segmental neurons, whereas in the centipede *Ethmostigmus rubipres* longitudinal connectives are pioneered from neurons in the brain that send their axons posteriorly to set up the parallel connectives. This difference between centipede and insect-crustacean ventral nervous systems is compounded by the fact that the pattern of segmental neurons in centipedes is quite different from that found in insects and crustaceans; centipede ganglia receive contributions from more widely distributed neurons, and there are more neurons in the centipede ventral cord when segmental axons are laid down. Comparisons of early neuronal outgrowth during embryonic development of the brain and thoracic ganglia also suggest a close affinity between crustaceans and insects (Harzsch et al., 1997; Therianos et al., 1995; Whitington et al., 1991). Paulus (1979) argued for arthropod monophyly on the basis of shared characters in the organization of photoreceptors and their satellite cells in compound and single-lens eyes. He further noted that insect and crustacean ommatidia, with their developmentally fixed numbers of cells, share more fine structural characters than either do with the chilopod ommatidia (which comprise an indeterminate number of elements).

Molecular Phylogenetic Studies of Arthropods

Molecular phylogenetic studies of the Arthropoda are summarized in Table 4. Field et al. (1988) sequenced a short segment of 18S rRNA but used representatives of just 10 phyla (only 4 of which were arthropods). Despite its limitations, the Field et al. work was pioneering. It was the first molecular phylogenetic study to test the monophyly of the arthropods, which it supported, and the work ini-

Table 4. Molecular views of monophyly within the arthropods.

Year	Author(s)	Arthropods monophyletic	Crustacea + Hexapoda	Myriapoda + Hexapoda (Tracheata)	Data
1988	Field et al.	Yes	Yes	No	18S rRNA
1989	Patterson	Yes	Yes	No	18S rRNA
1990	Lake	No	No	Yes	18S rRNA
1990	Field et al.	Yes	Yes	No	18S rRNA
1991	Turbeville et al.	Yes	Yes	No	18S rRNA
1992	Ballard et al.	Yes	Yes	No	12S rRNA (mitochondrial)
1992	Winnepenninckx et al.	Yes	Yes	n.a.	18S rDNA
1993	Van de Peer et al.	Yes	Yes	?	18S rDNA
1993	Wheeler et al.	Yes	Yes	No	18S rDNA + ubiquitin
1995	Winnepenninckx et al.	Yes	Yes	n.a.	18S rDNA
1995	Friedrich & Tautz	Yes	Yes	No	18S + 28S rDNA
1996	Garey et al.	Yes	Yes	n.a.	18S rDNA
1997	Regier & Shultz	Yes	Yes	No	EF-1a + POLII
1997	Eernisse	Yes	Yes	No	18S rDNA
1997	Spears & Abele	Yes	Yes	No	18S rDNA

tiated a stream of follow-up studies, continuing to use 18S rRNA sequences, and later the 18S rDNA gene itself. Each subsequent study has tended to use more taxa and longer nucleotide sequences for its data base, but until very recently most also continued to rely on the 18S gene. Problems associated with the 18S gene, use of short gene sequences, and single-gene phylogenetic inferences are well known and need not be repeated here. Furthermore, although there are now over 300 metazoan 18S sequences available, most published phylogenies have been based on fewer than 20 sequences (Eernisse, 1997). This is despite studies that suggest a minimum of 30–40 taxa are needed to accurately identify the root node of a large clade (Lecointre et al., 1993a, b; Sanderson, 1996; Hillis, 1996). In spite of methodological and sampling problems, recent molecular studies are beginning to converge on some similar conclusions. However, as Spears and Abele (1997) pointed out, ". . . in the crusade for understanding relationships among crustacean and other arthropod lineages, the rDNA data represent but a relic, and not the Holy Grail itself."

The most recent 18S sequence data suggest that insects share fewer similarities with the myriapods than they do with the Crustacea. Spears and Abele (1997) analyzed 31 18S sequences, and their results suggested that neither crustaceans nor insects were monophyletic. When they removed the "problematic" long-branched crustacean taxa (Remipedia, Cephalocarida, Mystacocarida), a myriapod + chelicerate clade emerged first, with insects as the sister group to a paraphyletic Crustacea. The Spears and Abele analysis also strongly supported malacostracan monophyly. Eernisse (1997) analyzed 103 sequences and concluded that (1) the Arthropoda are monophyletic, but only if the tardigrades are included [probably another 18S artifact], and (2) hexapods are more closely related to crustaceans than they are to myriapods. Regier and Shultz (1997) made a complete and welcome break with the 18S gene, using sequences from two other nuclear genes, the elongation factor (EF-1α) gene and the RNA polymerase II (POLII) gene. These trees were robust and mostly in agreement with the 18S work, concluding that: (1) Arthropods are monophyletic, (2) Crustacea are paraphyletic, and (3) insects are not the sister group of the myriapods, but arose from within the Crustacea.

Recent work by Boore et al. (1995) examined not gene sequences, but the linear arrangement of mitochondrial genes. This new type of data corroborates the gene sequence work and recognizes a mitochondrial gene arrangement that is unique to the crustaceans and insects alone.

In summary, the majority opinion from the molecular research, and the most recent opinions from both the morphological and molecular work, recognize four key features in arthropod phylogeny:

(1) Arthropods are monophyletic.
(2) Neither the Mandibulata nor the Atelocerata are natural groups.
(3) Crustaceans and insects constitute a sister group, exclusive of the myriapods.

(4) Crustacea are likely to constitute a paraphyletic taxon.

These last three conclusions are in conflict with 150 years of morphology-based thinking. Thus, two profound implications of these new studies are that the morphological attributes linking insects to myriapods might all be convergences (e.g., uniramous legs, tracheal system, Malpighian tubules), and that insects are actually "flying crustaceans" (in the same sense that birds are flying reptiles).

Emerging Views from Developmental Studies

The unique combination of segmentation and jointed appendages has allowed arthropods to develop modes of locomotion and feeding, and body region specialization, unavailable to other metazoan phyla. We now know that the fates of these segmental units and their appendages are under the ultimate orchestration of homeotic genes. These genes select the critical developmental pathways to be followed by cells during morphogenesis. Homeobox genes determine such basic body architecture as the dorso-ventral and the anterior-posterior body axes, where body appendages form, and the general types of appendages that form (Averof & Patel, 1997; Panganiban et al., 1997; Shubin et al., 1997). Homeobox genes can either suppress limb development, or modify it to create alternative appendage morphologies. A growing body of evidence suggests that these unique genes have probably played major roles in the evolution of new body plans among arthropods and the Metazoa in general (Davidson et al., 1995; Williams & Nagy, 1995; Panganiban et al., 1995).

The degree to which homeobox genes have been conserved is remarkable, and most of them probably date back at least to the Cambrian. For example, homologues of the *Pax-6* gene seem to dictate where eyes will develop in all animal phyla. *Pax-6* is so similar in protostomes (insects) and deuterostomes (mammals) that the genes can be experimentally interchanged and still function correctly. Homeobox genes modulate the expression of dozens of interacting, downstream, developmental genes whose products drive morphogenesis. The profound evolutionary potential of homeobox genes lies in this hierarchical nature. Variation in the output of these multigene networks can arise at many levels, simply by tinkering with the relative timing of gene expression—an evolutionary process we know as heterochrony. To understand the profound potential of homeobox genes to drive evolutionary change, consider that within the *Drosophila* genome 85–170 different genes might be regulated by the product of a single homeobox gene, the Ultrabithorax (*Ubx*) gene (Carroll, 1995).

A good example of the evolutionary potential of homeobox genes is seen in the abdominal limbs of insects. Abdominal limbs ("prolegs") occur on larvae of various insects in several orders, and they are ubiquitous in the order Lepidoptera, i.e., caterpillars. Abdominal limbs were almost certainly present in adult insect ancestors. Hence prolegs may have reappeared in such groups as the Lepidoptera through something as simple as the de-repression of an ancestral limb development program (i.e., they represent an atavism). We now know that proleg formation is initiated by a change in the regulation and expression of the *BX-C* gene complex (i.e., the Bithorax complex, which includes the *Hox* genes *Ubx*, *abdA*, and *abdB*) during embryogenesis (Carroll, 1995).

Molecular and developmental biology also seem to have broken the deadlock on the arguments over origins of uniramous and biramous limbs (e.g., Popadic et al., 1996; Panganiban et al., 1995, 1997; Shubin et al., 1997; Emerson & Schram, 1997). We now know that limb branching is a second-order phenomenon, probably orchestrated largely by the homeobox gene *Distal-less* (*Dll*). This single gene initiates development of unbranched limbs in insects and branched limbs in crustaceans. Antibodies that recognize *Dll* proteins show expression at the tips of insect limbs and also in biramous crustacean limbs (Panganiban et al., 1995). Branched limbs are formed when the gene is expressed ectopically in *Drosophila* (Diaz-Benjumea et al., 1994). In fact, *Dll* occurs in many animal phyla, where it is expressed at the tips of ectodermal body outgrowths in such different structures as the limbs of vertebrates, parapodia and antennae of polychaete worms, tube feet of echinoderms, siphons of tunicates, and appendages of arthropods. Furthermore, recent work suggests that whether an arthropod mandible is "whole-limb" (i.e., built of many segments) or "gnathobasic" (i.e., built of only the basalmost segments) also depends on the expression of the gene Distal-less. Thus, *Dll* is expressed in the whole limb (or multisegmented) jaws of myriapods, but not in the gnathobasic jaws of crustaceans and insects—still further testimony to the probable sister-group relationship of insects and Crustacea.

The Paleontological Data

Recent work has shown the fossil record of arthropods dates back to the early Cambrian, or perhaps the late Precambrian. And, by the mid-Paleo-

Table 5. Some important Precambrian and Cambrian arthropod *Lagerstätten* faunas.

Name	Age	Principal location
Orsten fauna	Upper Cambrian (~510 MYA)	Southern Sweden
Burgess Shale fauna	Middle Cambrian (~520 MYA)	British Columbia
Chengjiang fauna	Lower Cambrian (~530 MYA)	Southern China
Ediacaran fauna	Latest Precambrian (~560–600 MYA)	Ediacara Hills, Aust.

zoic, all five arthropod lineages were in existence and had already undergone substantial radiation. Arthropods are also the first land animals for which we have a geological record (Labandeira et al., 1988; Kukalová-Peck, 1990), and by the Late Silurian the first terrestrial scorpions and myriapods were already present. In fact, both terrestrial and marine myriapods have been reported from this period (Almond, 1985; Hahn et al., 1986; Labandeira et al., 1988), although molecular data suggest that myriapods might have arisen as early as the Cambrian (Friedrich & Tautz, 1995). The first centipede fossils occur in the Upper Silurian (~ 414 MYA) and, along with trigonotarbid arachnids, constitute the earliest known land animals (Jeram et al., 1990). The first millipede fossils occur in Devonian deposits (Almond, 1985; Robison, 1990); they are similar to the extant genus *Craterstigmus* (Shear et al., 1984) and are contemporaneous with the first terrestrial mites, pseudoscorpions, and scorpions (Størmer, 1969, 1977; Shear et al., 1987), as well as the first hexapods (Greenslade, 1988). The earliest known fossil hexapods are bristletails and collembolans from 390-million-year-old Gaspé mudstone (Labandeira et al., 1988). Some good records of these early creatures now exist, and the presence of these predatory arthropods suggests that complex terrestrial ecosystems were in place at least as early as the late Silurian. Perhaps the most important ancient arthropod fossils are those in which even the soft parts of the animal were preserved—the so-called ancient *Lagerstätten* (Table 5).

These ancient fossils have pushed the age of origin for the arthropods back to at least 600 MYA, and they provide us with critically important data on early arthropod anatomy and evolution. These extraordinary faunas are now telling us that Crustacea probably predate the appearance of trilobites in the fossil record, running counter to a long-held belief that trilobites were the most ancient arthropods. The recently exploited Chengjiang fauna of south China is Lower Cambrian, about 10 million years older than the Middle Cambrian Burgess Shale fauna (Chen et al., 1994). The Chengjiang fauna is very well preserved and includes at least 100 species of animals, many without hard skeletons, including the first known members of many modern groups. However, it is the arthropods that dominate this fauna, including trilobites and bradoriid "crustaceans" (and also tardigrades and onychophorans). The largest of the Chengjiang animals is *Anomalocaris*, also known from Ediacaran and Middle Cambrian deposits (Briggs, 1994). The Chengjiang fauna is very similar to that of the Burgess Shale, and it demonstrates that the arthropods were already far advanced by this early date.

The spectacular recent discovery by Klaus Müller and Dieter Walossek (Müller, 1983, 1992; Müller & Walossek, 1985; Müller et al., 1995; Walossek & Müller, 1992, 1997) of microscopic arthropods from the Upper Cambrian *Orsten* deposits of Sweden, has brought to light a rich fauna of minute crustaceans, crustacean larvae, and various crustacean-like arthropods. Among them, for example, is *Skara*, a cephalocarid-, or mystacocarid-like crustacean for which both naupliar larvae and adults have been recovered (the nauplius larvae are only a couple hundred microns long; adults are about 1 mm in length) (Müller & Walossek, 1986). *Skara*, and many other *Orsten* Crustacea, were surely meiofaunal animals not unlike modern marine meiofaunal crustaceans.

Fossils from this Cambrian site in Sweden have been collected since the days of Linnaeus, who actually described the first fossils from this area in 1757 (trilobites and conodonts). However, a brand-new kind of collecting began with Müller and Walossek's work in the 1980s. This new *Orsten* material is all microscopic, three-dimensional fossils. The *Orsten* arthropods show little or no signs of decomposition. They preserve details less than 1 micrometer in size (e.g., cuticular pores, the bristles on setae). Dozens of *Orsten* microcrustacea have so far been described. The recovery of these three-dimensionally preserved animals and the developmental series that have been found (with successive larval, juvenile, and adult instars—in animals less than 1 mm in length) have provided us with information on the detailed anatomy of body segments and appendages of many ancient stem-arthropods. The *Orsten* fauna shows that Cambrian Crustacea had all the attributes of modern crustaceans, such as com-

pound eyes, a head shield, naupliar larvae (with locomotory first antennae), and biramous appendages on the second and third head somites (the second antennae and mandibles).

Taken together, this recent paleontological work corroborates Whitington's observations long ago about the Burgess Shale fauna, that during Cambrian times the non-trilobite arthropods were both morphologically more varied and more numerous than were the trilobites (despite popular belief). We also now know that arthropods have probably been the dominant animals in terms of species diversity since the Cambrian. Arthropods comprise over one-third of all species described from Lower Cambrian strata.

Briggs and Fortey (1989) cladistically analyzed 23 of the Cambrian arthropod taxa, plus 5 extant groups. Their tree placed the Crustacea at the very base, as a paraphyletic sequence of taxa, and placed the trilobites and chelicerates near the top of the tree. The most recent molecular work does not conflict with this tree, in viewing the Crustacea as a paraphyletic group from which the other major arthropod clades emerged.

The Pentastomida

Pentastomids are obligatory parasites of vertebrate respiratory systems. There are about 100 described species, all of which infest various tetrapods, including two cosmopolitan species that infest humans. The blood-sucking adults inhabit respiratory tracts of their hosts, where they anchor themselves by means of their hooklike head appendages. For years it was believed that pentastomids were allied with the onychophorans as vermiform, pre-arthropod creatures. However, several recent molecular studies (using 18S gene sequences) have revealed the pentastomids to be highly modified crustaceans (Abele et al., 1989, 1992; Garey et al., 1996). Corroborative independent work over the past few years has come from cladistic analyses of sperm and larval morphology, nervous system anatomy, and cuticular fine structure (Wingstrand, 1972, 1978; Storch, 1984; Storch & Jamieson, 1992). Furthermore, Müller and Walossek's work on the Swedish *Orsten* fauna proves that the pentastomids (and also the tardigrades) had appeared at least by the Upper Cambrian, long before the land vertebrates had even evolved (Müller & Walossek, 1988; Walossek & Müller, 1994). So, we must ask what the original hosts of these parasitic crustaceans might have been. Walossek, Müller, and even Stephen Jay Gould have noted that Conodont fossils are common in all the Cambrian localities that have yielded pentastomids, and thus the conodonts (also long a mystery, but now widely regarded as parts of early fish-like vertebrates) might have been the original hosts of the pentastomids.

The Onychophora

As with pentastomids, onychophorans, too, were part of the amazing, early-Cambrian, explosive marine diversification. They have been found in Burgess Shale-type faunas at several localities, in Cambrian deposits from China and Siberia, and in the Swedish *Orsten* fauna (Xianguang & Weiguo, 1988; Xianguang & Junyuan, 1989; Ramsköld & Hou, 1991). And, we now know that Conway Morris's original reconstruction of *Hallucigenia* (from the Burgess Shale) had the animal turned upside-down. Ramsköld and Hou (1991) recently turned *Hallucigenia* over and found a second pair of legs, concluding it was an onychophoran with long dorsal spines. And there is now an onychophoran known from the Chengjiang deposits of China with side plates and spines (Ramsköld & Hou, 1991). *Aysheaia* (also from the Burgess Shale) was originally described by Walcott as an annelid, but it, too, is now regarded as an early marine onychophoran.

Conclusions

Let us now return to our two fundamental questions regarding arthropod evolution: Why are there so many arthropods, and what is the phylogenetic history of the arthropods? As to the first question, I propose six over-arching scenarios, each complex in its own right.

(1) The numerical superiority of arthropods is not a recent event. Recent fossil discoveries, and molecular clock data, inform us that arthropod diversification began very early in the history of the Metazoa, in the Precambrian, and by the Cambrian the arthropods were probably already the most speciose metazoan phylum on earth. Arthropods have been on a powerful phylogenetic trajectory for well over 600 MY. They have had a great deal of time to radiate, and with the exception of the trilobites and the eurypterids, all the major lineages have survived and continue to radiate.

(2) Their great size range, especially on the smaller end of the scale, adapts arthropods for a great variety of ecological niches. The Cambrian *Orsten* deposits tell us that a whole fauna of interstitial/meiofaunal arthropods already existed as early as the mid-Cambrian, and this habitat

has continued to be rich in adaptive radiation and specialized species ever since. Similar small-body-size niches are filled in a great many specialized environments today. We find high diversities of minute arthropods in habitats such as marine sediments, coral reefs, among the fronds of algae, on mosses and other primitive plants, and on the bodies of every kind of animal imaginable. There are even arthropod faunas that live strictly on the gills of other crustaceans (mites and small crustaceans). Small insects and mites have exploited virtually every terrestrial microhabitat available.

(3) The close relationship and coevolution with flowering plants (on land) and algae (in aquatic environments) have been a powerful force in the radiation of the arthropods. It is not just the insects that have been on a coevolutionary trajectory with plants—many crustaceans utilize algae as both a living substrate and a food source and show strong evidence of coevolution.

(4) The arthropods (insects) were the first flying animals, and the ability to fly led them into niches other invertebrates simply could not penetrate.

(5) Metamerism (the serially repeated body segments and appendages of arthropods) provides an enormous amount of easily manipulated body plan material upon which evolutionary processes can act. Given the great age, sheer diversity, and our emerging knowledge of regulatory genes in these animals, a high level of homoplasy is no longer surprising.

(6) The potential for major changes in body plans due to variations in homeobox genes, and the downstream genes they regulate, is just beginning to be realized, but this potential is clearly enormous. There seems little doubt that changes in homeotic genes over time have profoundly affected arthropod evolution. Considering the number and position of limbs in arthropods, and the flexibility of homeobox and regulatory switches, it is little wonder that arthropod anatomical diversity seems so endless.

As to the second question—what is the phylogenetic history of the Arthropods—it seems the plasticity of the arthropod body and homeobox gene expression may have produced an even higher level of homoplasy than once thought. As a result, some traditional morphological classifications are in conflict with molecular classifications. All the evidence suggests that the arthropods are monophyletic. However, fossil data, recent comparative neuroanatomical research, and molecular data all suggest that Crustacea are a paraphyletic group, and that the Crustacea and Insecta are very closely related to one another, but not to the Myriapoda. In fact, the insects appear to have arisen from within a crustacean stem line. Further, recent molecular and fossil data are beginning to suggest that the trilobites, chelicerates, insects, myriapods, and recent crustaceans *all* might have emerged from crustacean stem-line ancestors. This view of a paraphyletic Crustacea spinning off a series of other major arthropod lineages might explain why morphologists have been unable to come to agreement on the sister-group relationships of the major arthropod lineages. Resolution of this conflict will come, I predict, within the next two decades, with further understanding of the genetic regulation of developmental processes, examination of new nuclear and mitochondrial genes (and use of multiple gene data sets in phylogenetic analyses), and as more cladistic analyses include fossil species, particularly the growing series of Chengjiang, *Orsten*, and related arthropods.

A Speculation

The realization that insects might have arisen out of an ancestral crustacean stem line leads to many new implications concerning arthropod evolution. For example, given this scenario, one could search about among the Crustacea for a likely ancestor to the insects and in doing so recognize the presence of a "fixed" 19-segmented body plan in insects and certain crustaceans (or more likely a 20-segmented plan in each, Kukalová-Peck, 1991a, b; Scholtz et al., 1994; Scholtz, 1995). All insects are fixed on this body plan. Of all the crustacean higher taxa, this body plan consistently occurs only in the subclass Eumalacostraca—the crabs, shrimps, isopods, and their kin. Thus, if the insects did evolve from a crustacean ancestor, one might speculate that they could have evolved from a eumalacostracan. Examining the Eumalacostraca for a possible insect ancestry, there is only one group that is truly terrestrial, has evolved gas-exchange tracheae (granted, probably convergently to those of insects), has reduced/lost one pair of antennae (antennae one reduced in oniscideans, antennae two reduced in hexapods), and has strictly uniramous walking legs (as do the insects)—the terrestrial isopods (Isopoda: Oniscidea). Could it be that insects are not only flying crustaceans, but flying isopods?

The concept of a Eumalacostraca–Insecta sister-group relationship finds strong support in the comparative anatomy of arthropod central nervous systems. Development of the compound eye follows

similar morphogenetic events in insects and eumalacostracans (Hafner & Tokarski, in press). In addition, the optic lobes of pterygote insects and eumalacostracans are distinguished by nested retinotopic neuropils, each of which represents the whole eye. In these two taxa, these neuropils comprise an anatomically distinct lamina, medulla, and lobula complex (Strausfeld, 1996). The presence of these structures in pterygote insects and eumalacostracans was viewed as a homology indicating a sister-group relationship between these taxa by Osorio and Bacon (1994) and Nilsson and Osorio (1997). Further, eumalacostracans that have so far been examined also possess a distinctive form of neuron, called a bushy T-cell, which was first recognized in insects on the basis of its characteristic dendritic "tree" situated near the inner face of the medulla (Strausfeld, 1976). Bushy T-cells in insects and eumalacostracan crustaceans send their axons to large tangential dendrites that extend across substantial areas of the retinotopic mosaic. In those pterygote orders investigated, bushy T-cells comprise part of an evolutionarily conserved subset of retinotopic elements that contribute to elementary motion detector circuits (Strausfeld & Lee, 1991; Douglass & Strausfeld, 1995, 1996). The presence of these cell types in eumalacostracan crustaceans and pterygote insects implies that either identical circuits have arisen independently in the two groups, or the circuit for motion detection evolved in a common ancestor to insects and crustaceans has been maintained basically unchanged throughout the history of both groups. That the latter is more likely is suggested by the presence of small field retinotopic neurons that arise from the inner layer of the medulla of the apterygote *Thermobia* and extend into the lateral lobe of the protocerebrum (Strausfeld, 1998).

All crustacean nervous systems so far examined possess the architectonic and positional equivalent of a fan-shaped body, the neurons of which extend laterally into the protocerebral lobes, as they do in insects (Strausfeld, 1998). However, except in isopods there is no evidence in Crustacea of other components of the central body complex seen in insects. Further, in pterygote insects and isopods (but not in decapods or apterygotes) the fan-shaped body is supplied by a bridge of neuropil that lies posteriorly in the brain and connects the left and right protocerebral hemispheres. Strausfeld (1998) concluded that, while fan-shaped bodies are synapomorphic to insects and crustaceans, the protocerebral bridge may have evolved independently in insects and isopods.

Many morphological features are in conflict with a close malacostracan-insect relationship, including differences in tagmata arrangement and locations of the gonopores. The fossil record also does not support an isopod + insect sister-group relationship. The oldest known isopod fossils are only 300 million years in age (Phreatoicidea: *Hesslerella*, Carboniferous) (Brusca & Wilson, 1991). However, a recent analysis of phreatoicidean phylogeny suggests the isopods might have had their origin considerably earlier than this (Wilson & Keable, in press), and further examination of this unconventional idea may be warranted.

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SO MANY FISHES, SO LITTLE TIME: AN OVERVIEW OF RECENT ICHTHYOLOGICAL DISCOVERY IN CONTINENTAL WATERS[1]

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ABSTRACT

Although freshwaters contribute only about 0.01% to Earth's water supply, their fishes now number more than 10,000 species and thus account for at least 40% of all fish species. The continental fish faunas differ greatly in taxonomic composition and species richness, our state of knowledge of them, and the rate of discovery of unknown kinds. The ichthyofaunas of North America (about 1050 species), Europe (about 360), and Australia–New Guinea (about 500), are the most thoroughly documented, but new species continue to be described based on discovery of previously unseen forms and species-level taxonomic splits of known species. The ichthyofaunas of tropical Asia (perhaps > 3000), Africa (perhaps > 3000 species), and South and Central America (perhaps ≫ 5000 species), are species-rich yet incompletely known. Tropical freshwaters are the hot spots of recent and likely future ichthyological discoveries. Especially in the tropics, discoveries of species that signal new generic-level taxa are common, and new family-level groups are found occasionally. Everywhere ongoing phylogenetic studies often suggest or reveal unsuspected relationships. These are times of exciting discovery and advancement of knowledge in freshwater ichthyology. New discoveries beckon us to seek the many remaining unknowns in the diversity of life on our planet. These are also times of rapid and destructive change in freshwater habitats around the globe. These threats alert us to the increasing potential for permanent loss and ignorance of much of our planet's rich aquatic biota.

Key words: Actinopterygii, Characiformes, Craniata, Cypriniformes, freshwater, Gymnotiformes, ichthyofaunas, ichthyology, Otophysi, ray-finned fish, Siluriformes.

The species of craniate animals (hagfishes + vertebrates) are probably the most thoroughly documented of all the large clades in the tree of life. Yet new species of living craniates are described frequently, and most of these are fishes. In a recent tally, Eschmeyer (1998) found that about 200 fish species are described annually.

Fishes are water-dwelling craniates with permanent gills borne on the walls of pharyngeal arches or pouches. Fishes have median fins supported by cartilaginous or bony rays, and most have paired fins, but never limbs bearing digits (Bond, 1996; Helfman et al., 1997). Uniquely, fishes are studied by ichthyologists. Characterized in this way, the total number of living fish species is about 25,000 and accounts for roughly 50% of the extant species richness of the Craniata, while the other half are tetrapods (Nelson, 1994). Thus delimited, fishes are not a monophyletic group because their subgroups are arranged along a phylogenetic "ladder" below the tetrapods (Fig. 1). The vast majority of fish species, however, belong to one clade: the Actinopterygii or ray-finned fishes. There are at least 23,700 living actinopterygian species (Nelson, 1994). The species richness of the ray-finned fishes strongly contrasts with the present species poverty of the other higher fish clades (Fig. 1).

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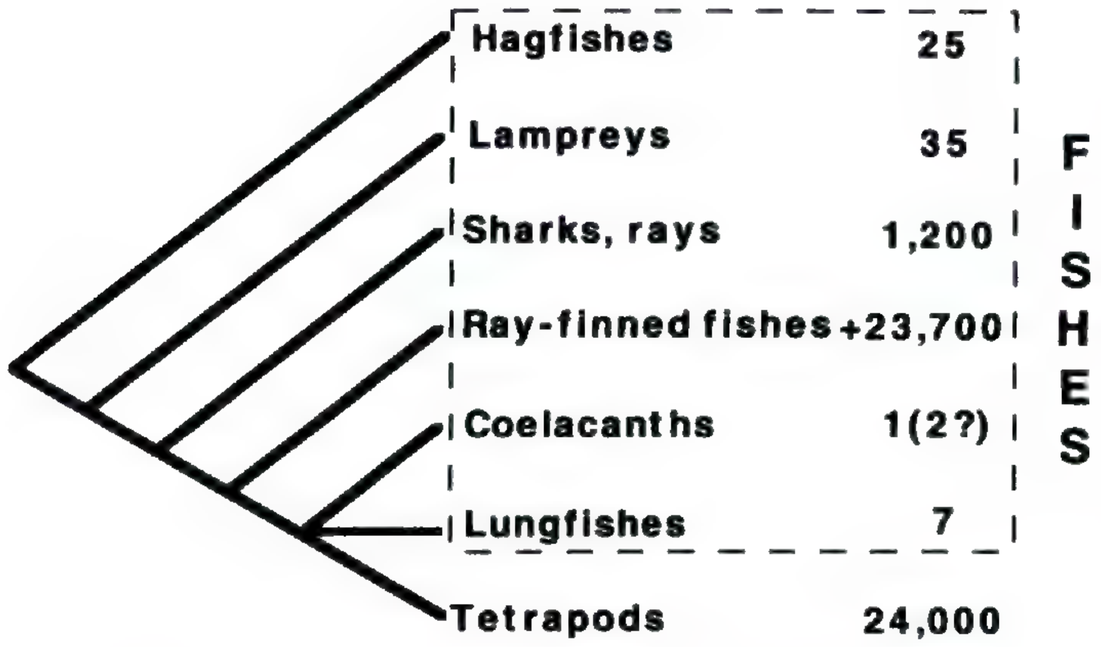


Figure 1. The phylogenetic tree of the major groups of living craniate animals and estimated number of extant species in each (based on Nelson, 1994, and Compagno, pers. comm.).

The large-scale ecological distribution of fishes is strongly bimodal: 58% of fish species are marine, 41% (about 10,000 species) live principally in freshwater, and only about 160 (~ 1%) regularly migrate between salt and fresh water (Cohen, 1970; McDowell, 1988). This diversity of freshwater fishes causes some pause in light of the impressive fact that freshwater makes up a tiny amount, only about 0.01%, of Earth's water supply. Thus, Horn (1972) calculated that freshwaters hold a far greater "density" of fish species than the oceans—greater by 7500 times! But, of course, most marine species live in a relatively small volume of seawater in the productive photic zone, especially around coral reefs.

In this paper we focus on the diversity of freshwater fishes, those living in continental lakes, rivers, streams, and swamps. In addition to their great species numbers, freshwater fishes are interesting to us because of their tremendous variety in form, function, and habit. Freshwater fishes provide evolutionary biologists with some of the best examples of natural selection and adaptation, e.g., guppies (Endler, 1983) and sticklebacks (Bell & Foster, 1994), divergence and speciation, e.g., Laurentian Great Lakes whitefishes (Smith & Todd, 1984) and North American Great Basin fishes (Hubbs & Miller, 1948; G. R. Smith et al., in press), reticulate evolution, e.g., Catostomidae (Uyeno & Smith, 1972), species flocks (African cichlids, Stiassny, this paper), and Lake Titicaca *Orestias* (Parenti, 1984a; Costa, 1997), and the historical development of biotas on scales from regional, e.g., postglacial age North America (Bailey & Smith, 1981) to continental, e.g., North America (Patterson, 1981; Grande, 1994) and Africa–South America (Lundberg, 1993).

Here we are particularly interested in the expanding knowledge of freshwater fishes: the amounts, natures, and sources of recent ichthyological discoveries and prospects for the future. Our first concern is with the new species of freshwater fishes that are being found and described. In addition, we call attention to discoveries of other important aspects of fish diversity: new phylogenetic lines and unexpected relationships, newly found extraordinary characteristics of phenotypes and natural histories, and new understanding of the processes that control rates of speciation and extinction. The major sources of previously unseen fishes are explorations in biotically uncharted waters, especially in remote tropical areas, and in difficult-to-sample aquatic habitats such as deep river channels, cataracts, and leaf litter. Yet survey work at long-visited sites, such as the African Great Lakes and Amazon margins, continues to yield many new species. Unrecognized and even formerly unseen species are routinely found in the course of thorough taxonomic studies, especially in revisions of species-rich, freshwater groups of ostariophysans, atherinomorphs, and percomorphs. Another source of recent species descriptions involves taxonomic concepts and practice. Application of species criteria that emphasize diagnosability or distinctness of populations over traditional geographically widespread, polytypic species results in an increase of recognized species.

Following some additional background information aimed mainly at the non-specialist, we take a regional approach to showcasing freshwater fish diversity and recent discoveries. Based on our individual expertise, authorship responsibility follows geography: Smith—North America, Lundberg—South and Central America, Kottelat—Europe and tropical Asia, Stiassny—Africa, Gill—Australia and New Guinea.

Freshwater Fish Faunas: Taxonomic Composition

Most higher fish clades include species that sometimes or permanently live in freshwater. All lamprey species reproduce in freshwater; some remain there whereas others migrate to the sea to feed. Modern lungfishes are restricted to freshwater. Several species of sharks, sawfishes, and rays regularly enter freshwater, and the Potamotrygonidae are a moderately diverse clade of sting rays confined to South American rivers and lakes. Scores of actinopterygians live in freshwater. Familiar examples of these are sturgeons, gars, carps, piranhas, tetras, electric eels, catfishes, pikes, trouts, guppies, sticklebacks, black basses, sunfishes, darters, cichlids, bettas, and gouramies. If biotic

success is measured by species numbers and breadth of overall distribution, the dominant group of freshwater fishes is the Otophysi (Ostariophysi in older literature). Otophysi contain four monophyletic taxa ranked as orders: (1) Cypriniformes (carps, minnows, barbs, suckers, loaches) of North America, Europe, Asia and Africa, presently with roughly 2700 species; (2) Characiformes (tetras, piranhas), now with at least 1300 species, distributed today in the Neotropics and Africa, and formerly extending to Europe and the Arabian Peninsula; (3) Gymnotiformes (electric eel and knifefishes) of South America with over 90 described species; (4) Siluriformes (catfishes) currently with more than 1400 species and an overall distribution including all continents, even Antarctica as recorded by fossils from Seymour Island (Grande & Eastman, 1986).

Biogeography and Physiological Ecology of Fishes

Freshwater fishes play important roles in a variety of biogeographic studies. Some are endemic markers that delimit regional biotas. Fish species are censused in determinations of regional and global patterns of species richness or biodiversity. Each clade of fishes has its singular biogeographic history. Many freshwater fish clades are components of biotas that share with others common vicariant or dispersal-based biogeographic histories. Many fish species and higher taxa serve as biotic indicators of former connections among regions from watersheds to continents.

Monophyletic groups of strictly freshwater fishes have special significance in continental biogeography because they require freshwater for dispersal, and thus their distributions are correlated with the evolution of topography and watersheds. The degree to which fishes are physiologically and behaviorally restricted to freshwater varies widely. Species and higher taxa of fishes are commonly grouped according to their observed present and presumed historical habitat and physiological salinity tolerances (Myers, 1938; Darlington, 1957). So-called "primary" freshwater fish groups, such as most otophysans, spend their entire lives in freshwater habitats and are physiologically incapable of coping with seawater. It is important to know the phylogenetic level at which subgroups within a clade show strict primary freshwater distributions (Patterson, 1975; Lundberg, 1993).

"Secondary" freshwater fish taxa, such as cyprinodontiforms and cichlids, are usually limited to freshwater, especially for reproduction, and have

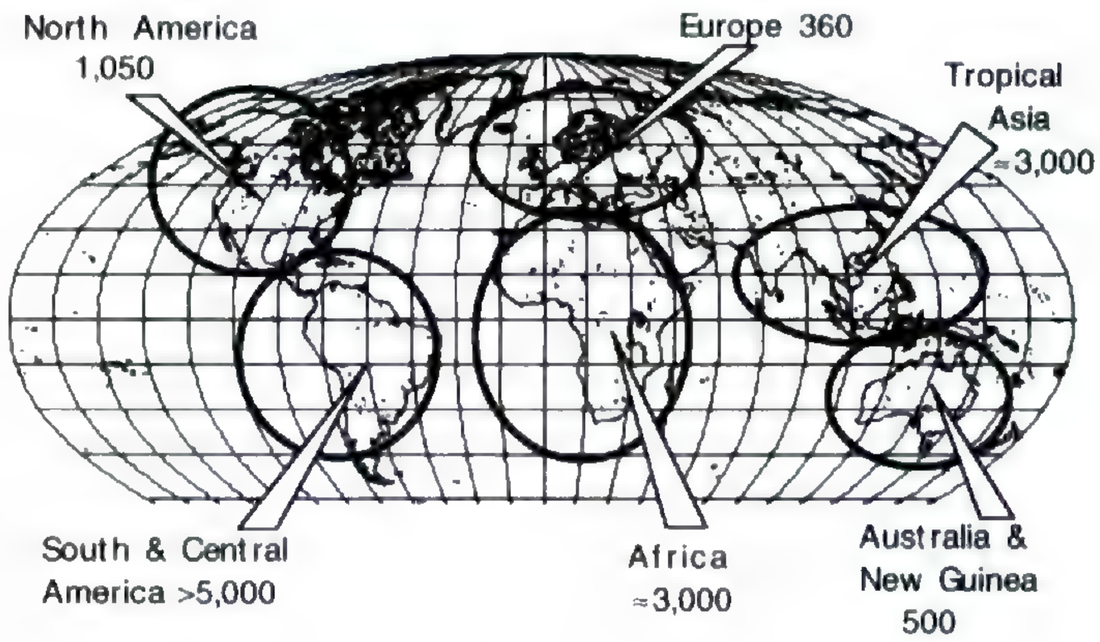


Figure 2. Estimated number of living fish species in the major freshwater faunas. Base map created by Xerox Corporation's Palo Alto Research Center, Map Viewer.

their greatest diversity and abundance there, but occasional individuals or member species may be found regularly in coastal saltwater habitats. Secondary freshwater fishes might, therefore, disperse through the sea. "Peripheral" fishes are marine groups that include species with individuals that may move sporadically into freshwater or species that are permanent freshwater residents; common examples include herrings, anchovies, needlefishes, puffers, pipefishes, drums, gobioids, and soles. All continental ichthyofaunas include peripheral groups with persistent freshwater populations, and the freshwater faunas of many islands comprise entirely peripheral fishes. These are generally regarded as "marine dispersants" but, unless or even if their closest intragroup relatives are known to be resident marine taxa, peripheral fishes should not automatically be ignored in comparing intercontinental faunas.

Distinctness of Freshwater Ichthyofaunas

At the scale of continents (and Alfred Wallace's Zoogeographic Realms) ichthyofaunas differ greatly in their taxonomic composition and species richness (Fig. 2). Australia and Europe each have hundreds of fish species, North America has somewhat more than 1000, tropical Asia and Africa each have about 3000, and there may be more than 5000 fish species in the American tropics. Especially at the species level, there are also major differences in thoroughness of our knowledge of continental fish faunas. Higher-level taxonomic differences are mostly caused by the long and distant isolation of the freshwaters of most continents. Differences in species diversity probably have more to do with factors that control both terms of biological diversification: speciation and extinction. Differences in the extent of knowledge of species result from different histories of exploration and study, as well as species richness. It is not surprising that we know

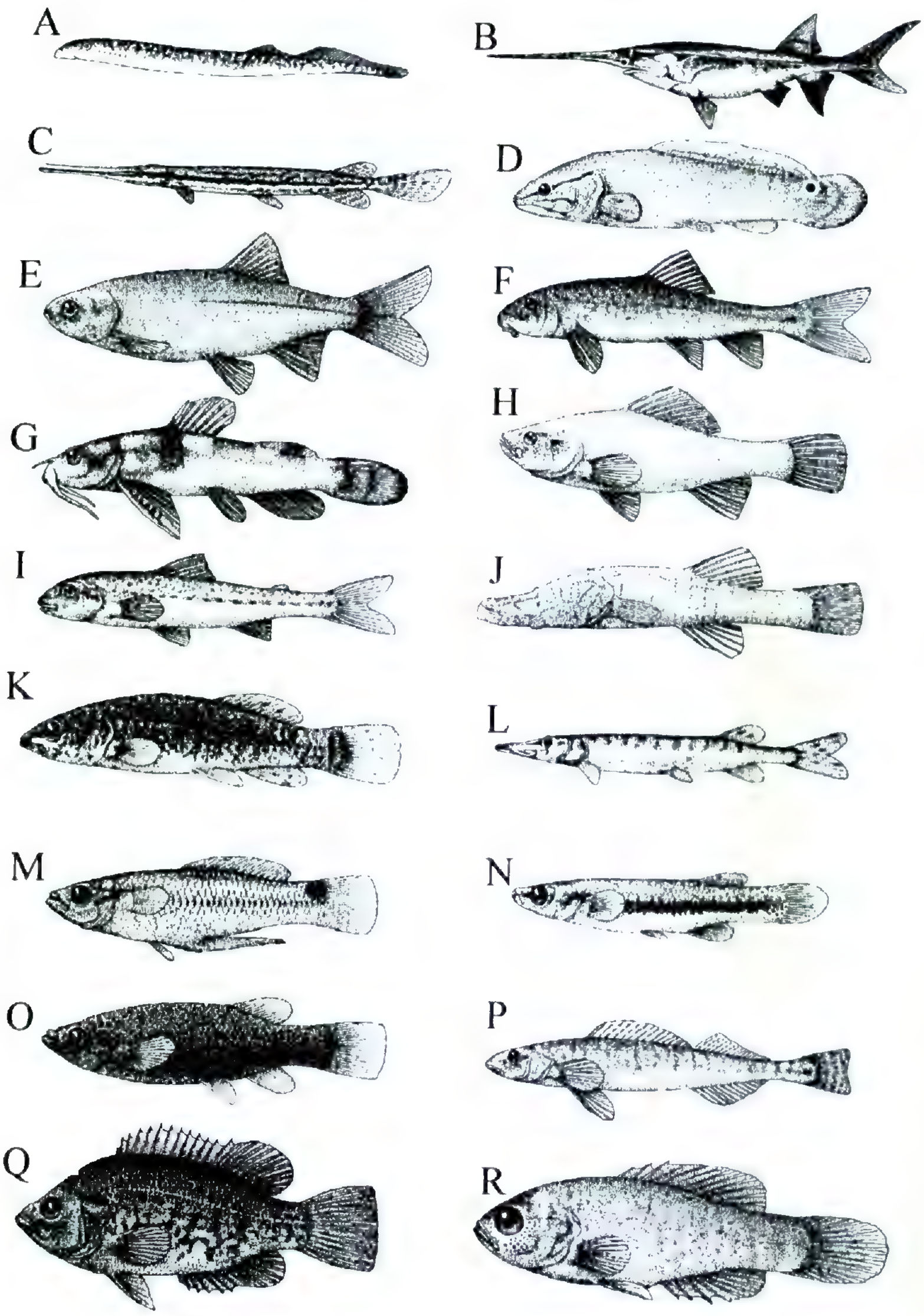


Figure 3. Fish icons of North America. —A. *Petromyzon marinus*, sea lamprey. —B. *Polyodon spathula*, paddlefish. —C. *Lepisosteus osseus*, longnose gar. —D. *Amia calva*, bowfin. —E. *Larinia exilicauda*, hitch. —F. *Catostomus platyrhynchus*, mountain sucker. —G. *Noturus furiosus*, Carolina madtom. —H. *Aphredoderus sayanus*, pirate perch. —I. *Percopsis omiscomaycus*, trout-perch. —J. *Speoplatyrhinus poulsoni*, Alabama cavefish. —K. *Umbra limi*, central mudminnow. —L. *Esox masquinongy*, muskellunge. —M. *Heterandria bimaculata*, spottail killifish. —N. *Fundulus notatus*, blackstripe topminnow. —O. *Goodea atripinnis*, blackfin goodea. —P. *Percina caprodes*, logperch. —Q. *Archoplites interruptus*, Sacramento perch. —R. *Elassoma evergladei*, Everglades pygmy sunfish. Drawn by John Megahan.

more about the less diverse European, North American, and Australian faunas, than the rich African, tropical Asian, and South American assemblages.

NORTH AMERICA

The North American freshwater fish fauna occupies the Nearctic Realm: from Canada and Alaska, south to the Transvolcanic Axis south of the Mexican Plateau (Fig. 2). Over 1050 species (e.g., Fig. 3) in about 175 genera and 32 primarily freshwater families (plus a few freshwater species belonging to 35 genera in 24 mostly marine families) occupy rivers and lakes currently or recently draining to the Arctic, eastern Pacific, Caribbean, and western Atlantic. The numerically dominant families are: Cyprinidae (305 species), Percidae (172), Poeciliidae (75), Catostomidae (68), Ictaluridae (48), Goodeidae (40), Fundulidae (37), Centrarchidae (32), Atherinidae (35), Cottidae (27), and Cichlidae (21). These 11 families make up about 80% of the species in the fauna.

Historical biogeography of the fauna was sum-

marized in the comprehensive treatise *Systematics, Historical Ecology, and North American Freshwater Fishes* edited by Mayden (1992). Regional biogeography was thoroughly analyzed in *Zoogeography of North American Freshwater Fishes* edited by Hocutt and Wiley (1986). Systematic accounts and distributional records have been documented in about four dozen regional, provincial, or state fish monographs and the *Atlas of North American Freshwater Fishes* (Lee et al., 1980). Nine families and 128 genera are now endemic to North America, but some (e.g., Amiidae, Hiodontidae) were more widespread in the past. The principal intercontinental relationships of most of the fauna (e.g., Cyprinidae, Catostomidae, Salmonidae, Esocidae, Percidae) are with Eurasia, whereas the Characidae, Pimelodidae, Cyprinodontidae, Fundulidae, Profundulidae, Poeciliidae, and Cichlidae have neotropical relationships (Patterson, 1981; Grande, 1994).

The combined fauna of Atlantic and Gulf of Mexico drainages is several times as diverse as the Pacific drainage fauna because its land area is larger and has been geologically and climatically more stable during the Cenozoic. The Mississippi Basin is the center of North American freshwater fish diversity; its fauna is the largest with 375 species. The Eastern Highlands, mostly in the Mississippi drainage, have the most distinctive fishes with 57 species (Mayden, 1985, 1987). Diversity decreases with distance away from the Mississippi Basin heartland. The vast northern glaciated areas are depauperate because of long winters and slow postglacial recolonization (Fig. 4; see also C. L. Smith, 1985). Western and southern faunas are depauperate because they are mountainous, arid, and isolated by barriers. The Great Basin (Hubbs & Miller, 1948; Hubbs et al., 1974) and Colorado River faunas, in western North America, have the lowest diversity, but highest endemism (Miller, 1959), over 50%, indicating a high extinction rate (G. R. Smith et al., in press).

Freshwaters of the United States were thoroughly explored in the 19th century and the first half of the 20th century; North America now has few undiscovered species except in Mexico and Central America. Recently described additions to the North American fauna are mostly allopatric, divergent local populations of darters, minnows, and suckers that are elevated to species status based on the phylogenetic and evolutionary species concepts (Mayden & Wood, 1995). An unusual recent discovery was *Scaphirhynchus suttkusi* Williams & Clemmer, a new species of sturgeon from the Mobile Basin. Molecular data have also revealed diverse populations of salmons, trouts, and whitefish-

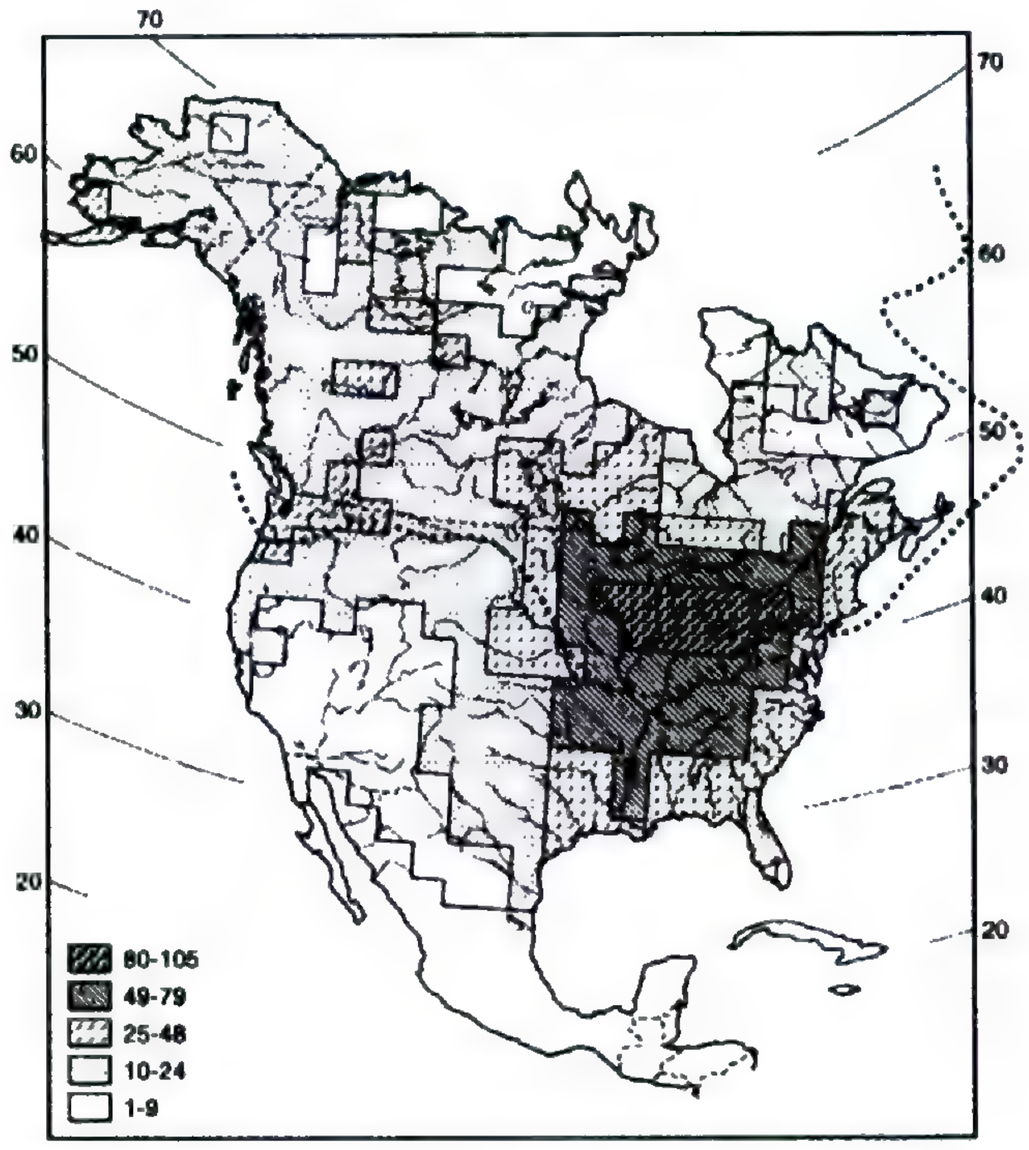


Figure 4. Fish species diversity gradients displayed as number of species per quadrat, considering only those portions of the fauna that have successfully invaded glaciated regions in eastern North America, or survived in the regions of montane glaciation pluvial lakes in the West. From dark to light the shading levels indicate species richness from 80 to 105; 49 to 79; 25 to 48; 10 to 24; 1 to 9.

es (Bernatchez & Wilson, 1998), some of which are sympatric but ecologically different. Some of these forms fit species concepts based on inferred genetic and ecological differences between populations that spawn in different times and places. With emphasis on historical processes of origin, however, G. R. Smith et al. (1995) defined species as lineages separated from each other by genetically based morphological, reproductive, ecological, or behavioral barriers sufficient to confer long-term historical independence.

Monographs on groups of the North American freshwater fish fauna have been produced recently. The most magnificent are *The Evolutionary Biology of the Threespine Stickleback*, edited by Bell and Foster (1994), and *Native Trout of Western North America*, by Behnke (1992). Two of North America's famous relictual fishes and their fossil relatives, paddlefishes (Polyodontidae) and bowfins (Amiidae), are comprehensively treated by Grande and Bemis (1991, 1998). Also notable as popular as well as scientifically valuable treatments are *The Handbook of Darters* by Page (1983) and *The American Darters* by Kuehne and Barbour (1983). The fauna has also benefited from outstanding ecological and evolutionary treatises, such as that by Matthews and Heins (1987). The field eagerly awaits

the appearance of the *Freshwater Fishes of Mexico*, in preparation by Robert R. Miller.

Because North American fish species are well described, the most exciting recent discoveries are of evolutionary relationships and patterns. New insights on relationships are the result of research in molecular and morphological phylogeny, an area of systematics substantively pioneered by North American ichthyologists (Wiley, 1981; Burr & Mayden, 1992). Systematic summaries of most of the important groups can be found in Mayden (1992). The higher-level systematics of representative fish taxa is treated in Stiassny et al. (1996). Nineteen of the 21 richest North American families have been the object of substantial phylogenetic work (Burr & Mayden, 1992: 32). More than 250 phylogenetic papers on North American fishes have been published, but much more cladistic work is needed (Burr & Mayden, 1992: 59). Unexpected recent discoveries include the close relationship of the live-bearing Goodeidae (Fig. 3O) of the Mexican Plateau to the egg-laying *Crenichthys* and *Empetrichthys* of the Great Basin (Parenti, 1981; Webb, 1998) and the relationship of the pygmy sunfishes, *Elassoma* (Fig. 3R), to the sticklebacks (Johnson & Springer, 1997). Other relationships, based on DNA sequences, include spinedace (Hubbs & Miller, 1960) and creek chubs among cyprinids (Simons & Mayden, 1997), and Pacific trouts and salmons among salmonids (Stearley & Smith, 1993; Philips & Oakley, 1997). In addition, studies of phylogeny have revealed the frequent influence of introgression on evolutionary patterns of North American freshwater fishes (G. R. Smith, 1992; Dowling & Secor, 1997).

New evolutionary analyses relate patterns in fish diversity through time to the geological control of rates of evolution and extinction. In tectonically fragmented and volcanically disturbed areas, high extinction rates control diversity. Comparison of fossil and Recent North American fishes suggests that they have not evolved substantially in response to Pleistocene environmental changes. These results are consistent with those based on discovery of early to middle Cenozoic fishes closely related and similar to extant species in North America and South America (Cavender, 1986; Wilson & Williams, 1992; Lundberg, 1998). Molecular and morphological data in their geological context indicate that anagenic change and speciation are much slower than geological and climatic changes (G. R. Smith et al., in press). These results suggest that the current (latest of the past two dozen or so cycles) post-glacial species assemblages in glaciated regions, such as the Great Lakes basin, are not likely to be ecological communities with fine-tuned interspecific interactions.

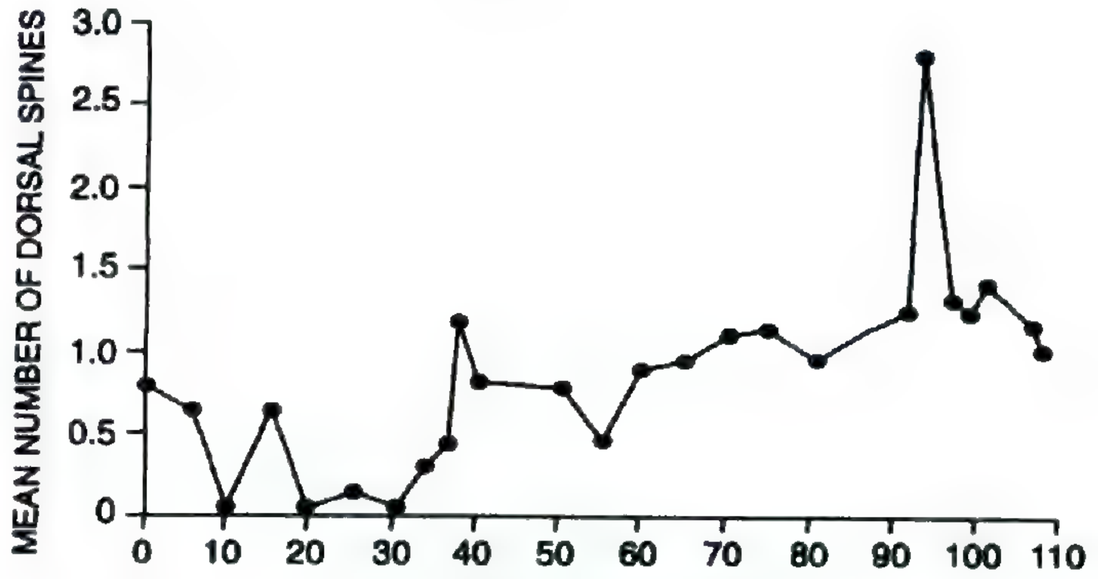


Figure 5. Short-term fluctuations within a 100,000-yr. trend in stickleback spines (from Bell et al., 1985) demonstrating limited significance of rapid changes over the long course of phenotypic evolution.

Studies of rates of fish evolution were pioneered by Hubbs and Miller (1948) in the Great Basin. They documented rapid evolution based on observed changes attributed to post-pluvial isolation (in the past 10,000 years) of populations assumed to have been uniform when waters were connected in pluvial times (e.g., Kocher & Stepien, 1997). Current studies negate the assumption of genetically uniform species (G. R. Smith et al., in press). Studies of Pliocene and Pleistocene morphological changes in the Great Basin show that the early, rapid responses to environmental change such as those documented by Hubbs and Miller (1948, 1974) do not usually lead to new species (Bell et al., 1985; G. R. Smith et al., in press). The isolation of small populations in rapidly changing environments promotes rapid changes, but over the long term these appear to be short-term fluctuations within slow trends (Fig. 5; Bell & Haglund, 1982; Bell et al., 1985).

Fossil discoveries suggest that the modern North American families probably date back more than 65 million years to the Cretaceous, most genera to the Miocene, and species mostly to the Pliocene or early Pleistocene. Species formation averages slower than one branch per million years per clade in rivers (depending on the family) but may be more rapid in lakes (Echelle & Kornfield, 1984). Molecular evolution (Kocher & Stepien, 1997) may be stochastically constant within 10–25% error; for certain mitochondrial genes, it varies from 0.5% sequence divergence per million years in salmonids to about 1%/m.y. in cyprinids and cyprinodontoids (Fig. 6; G. R. Smith et al., in press). The above estimates suggest that adaptive evolutionary responses to the current global ecological crisis are unlikely.

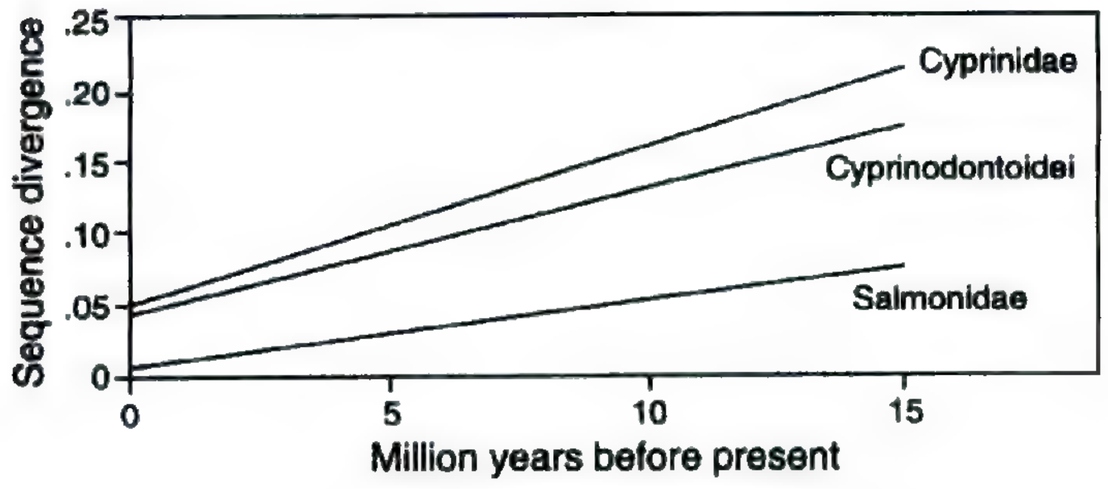


Figure 6. Estimated rates of mitochondrial gene sequence divergence vary from 0.5% to 1% per million years among salmonids, cyprinids, and cyprinodontoids. Fossil discoveries enable calculation of these rates when synapomorphies of earliest known fossils can be used to identify the internode segment or part of the lineage in which the fossil fish was a member. The age of the fossil can then be used to provide a minimum estimate of the age in millions of years—the denominator in the rate equation. The estimated sequence divergence between taxa for which an age estimate is available then becomes the numerator in the rate equation. The rates are then used to estimate the ages of other vicariance barriers and ages of groups.

Prospects for future ichthyological discoveries in North America include some new species in Mexico and important advances in our knowledge of patterns and processes of fish diversification.

SOUTH AND CENTRAL AMERICA

The vast Neotropical ichthyofauna, estimated to contain between 5000 and 8000 species (Schaefer, 1998; Vari & Malabarba, 1998), inhabits the freshwaters of South and Central America (Fig. 2), with a handful of cichlids, pimelodid catfishes, and characids extending north into Mexico or the southernmost U.S. The great majority of Neotropical fishes belong to one of five dominant groups: characiforms, siluriforms, gymnotiforms, cyprinodontiforms, or cichlids.

Within the Neotropics the Amazon Basin contains Earth's most diverse riverine fish fauna that certainly far exceeds 1000 species. The Orinoco, Paraná, and other large, tropical rivers flowing to the Atlantic are also species-rich. Fish diversity drops sharply in the watersheds emptying into the Caribbean and Pacific, and southward into temperate South America where the taxonomic composition also changes markedly. Central American freshwaters contain roughly 300 species (Bussing, 1976). In this region the San Juan Basin of Nicaragua and Costa Rica has the most diverse fauna with about 54 species (Bussing, 1985).

Neotropical cichlids and gymnotiforms are endemic, monophyletic clades. On the other hand, Neotropical characiforms (Vari & Malabarba, 1998), siluriforms (de Pinna, 1998), and cyprinodontiforms (Costa, 1998) each contain several separately monophyletic subgroups with incompletely known extralimital relationships. Some Neotropical fish clades have their closest relatives of today in African freshwaters (Lundberg, 1993; Vari & Malabarba, 1998; de Pinna, 1998): lungfish, arapaima, ctenoluciid + erythrinid and some characid characiforms, doradoid catfishes, aplocheiloid and poeciliid cyprinodontiforms, cichlids, and nandids. A few of the Neotropical cyprinodontiforms are most closely related to North American taxa (Parenti, 1981). Many Neotropical fishes, including individual species and some small clades, have their proximate relatives and presumed ancestry in coastal marine waters, e.g., river sting rays, various herrings and anchovies, drums, soles, needlefishes, toadfishes, and a puffer.

It is scarcely surprising that no comprehensive treatise yet exists for the Neotropical fish fauna. However, two recent publications have immensely advanced access to information about the fauna. The massive *Catalog of Fishes* (Eschmeyer, 1998) provides the most thorough listing ever retrieved of the binomials applied to all fishes and also contains an extensive bibliography of descriptive ichthyology. The electronic version of the *Catalog* has greatly facilitated an estimate of the historical accounting of published description of Neotropical fishes. The symposium volume *Phylogeny and Classification of Neotropical Fishes* (Malabarba et al., 1998) contains 28 papers that summarize much up-to-date systematic knowledge of higher Neotropical taxa.

The discovery of Neotropical fish species became an active enterprise by about 1825 and it continues at a high rate. The latest ca. 50-year trends in species description for the Neotropical siluriforms, gymnotiforms, characiforms, cyprinodontoids, and cichlids are shown in Figure 7 (data from Eschmeyer, 1998). Overall about 1400 species were described during this period, and the vast majority of these are considered valid. Using the recent estimates for total Neotropical fish species richness, this amount of discovery and description could represent about 25% of the whole ichthyofauna.

The levels of recent discovery and estimates of total species richness for the five major groups are truly impressive. The siluriforms are the richest of the major Neotropical groups with at least 1400 species (Nelson, 1994). Between 1950 and 1997, 491 Neotropical catfishes were described, and more than half of these were published in the last 20 years. Several known catfish species await description, and certainly other unrecognized and unseen species will turn up in natural history collections

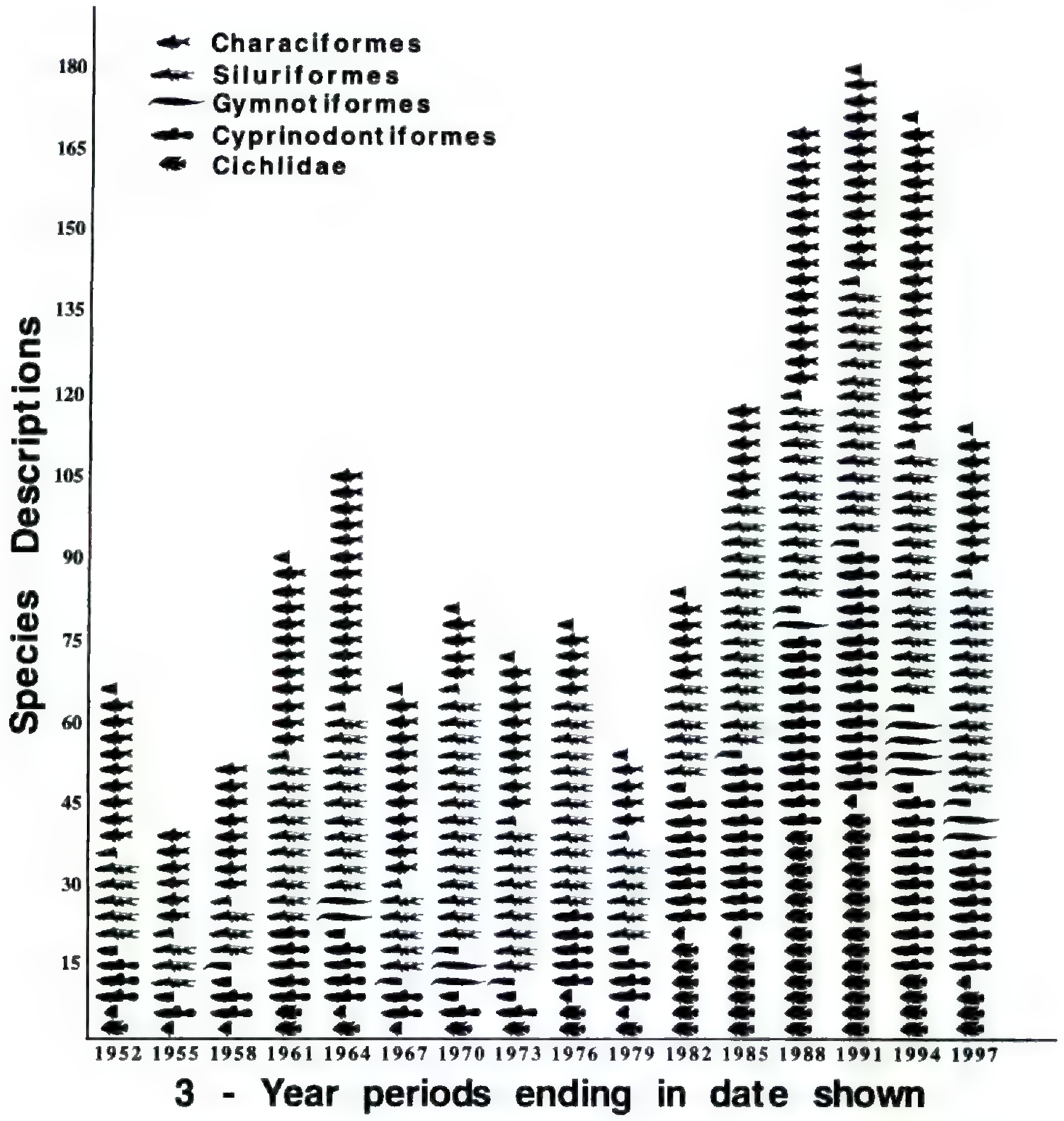


Figure 7. Neotropical fish species descriptions 1950–1997 for characiforms, siluriforms, gymnotiforms, cyprinodontiforms, and cichlids based on data from Eschmeyer (1998). Each whole fish icon represents three descriptions, partial fish icons equal one or two descriptions; each column covers a three-year period.

and new field samples. Thus, any estimate of the total species richness of Neotropical catfishes is now imprecise, but close to or more than 2000 species should be expected.

Mago-Leccia (1994) recognized 94 valid species of electric fishes; Campos da Paz and Albert (1998) estimated the total to exceed 100 species. In just the last 25 years, descriptions of 31 new gymnotiform species and 13 new genera were published, and we are aware of an additional 32 undescribed species. As with catfishes, it is difficult to estimate the total species richness of Neotropical characiforms because of their taxonomic complexity and the high rate of discovery of undescribed species. Nelson (1994) estimated an overall total of 1300 characiform species, and Vari (1998) predicted that far greater than 2000 exist overall. Of these only about 225 are African. Between 1950 and 1997, over 450 characiform species were described in the Neotropics, and although annual rates of description vary, there is no indication of reduction. Experts working with characiform taxonomy are aware of many dozens of undescribed forms.

South and Central American cyprinodontiforms are estimated at about 375 species (Huber, 1998; Nelson, 1994). From the Neotropics 203 cyprinodont species have been described since 1950, and of these over 80% (166) were published after 1974. These numbers would be higher if new species in shared and closely related genera from Mexico, the U.S., and the West Indies were included. Neotropical cichlids are not as species diverse as African cichlids, but their numbers are high and increasing. Kullander's current (1998) estimate is that the total will be about 450 Neotropical cichlids, of which more than 100 are undescribed. About 30% (134) of the estimated total have been described since 1974.

The continuing high level of discovery of fish species in the Neotropics may be explained by the combination of an increased amount of field and taxonomic work applied to a very large, taxonomically complex, and undersampled fauna (see also Vari & Malabarba, 1998). We could cite dozens of recent examples of ichthyological discovery coming out of small and incidental to large, focused field exploration throughout the Neotropics. However, the recent history of ichthyological exploration in Venezuela illustrates the great magnitude of discovery, on one large, hydrographically complex re-

gional scale, that is still possible in many areas of South America. About 30 years ago Mago-Leccia (1970) published the first comprehensive list of Venezuelan freshwater fishes, which included 474 species. In 1997 Taphorn et al. were able to revise the Venezuelan list upward to 1065 species (a 125% increase) based largely on the combined results of much survey work carried out during the intervening approximately 25 years.

The number of taxonomists involved in describing Neotropical fishes has increased over the years, although we may have reached a plateau. Using characiform taxonomy as an example, for the 10 quarter-century periods between 1758 and 1990 the numbers of authors of species descriptions were 4, 5, 9, 18, 12, 31, 28, 38, 64, 57, and in the 7 years since 1990 already 54 authors have been involved in describing characiforms. Between 1970 and 1997, 228 authors contributed to papers describing approximately 960 species of Neotropical characiforms, siluriforms, gymnotiforms, cyprinodontiforms, and cichlids. As expected, authorship productivity of species descriptions varies tremendously: the range of descriptions across author is 1–102, with a strong mode of 1 description per author, and a median of 2. Seven highly productive individuals were sole or co-authors of at least 40 species descriptions and, remarkably, the total contribution of these 7 persons accounts for over half (524) of the 960 recent descriptions.

A general pattern of biodiversity is that species richness increases with sample size, e.g., with area, time, specimens, taxonomic scope, etc. The effect of taxonomic scope on Neotropical fish diversity is evident at different ranks. For example, differences in the amounts of species discovery among catfish families are correlated with the species richness of those families (Fig. 8). The same relationship appears to hold at the genus level with the very large genera *Corydoras, Aspidoras, Trichomycterus,* and *Hypostomus* accounting for over 30% of all Neotropical siluriform species described during the last ca. 25 years. Among characiforms, too, the large genera *Leporinus, Hyphessobrycon, Cyphocharax, Curimata, Astyanax,* and *Creagrutus* account for about 30% of the species described in the same period.

Exemplifying the great yield of new species that can be found by in-depth study of relatively small-bodied characiforms, Vari and Harold (1998) have raised the species number of *Creagrutus* (east of the Andes) from 15 to 56, a 373% increase. Revisionary work at the species level seems more often than not to produce a net increase in the number of Neotropical fish species. Kullander (1980),

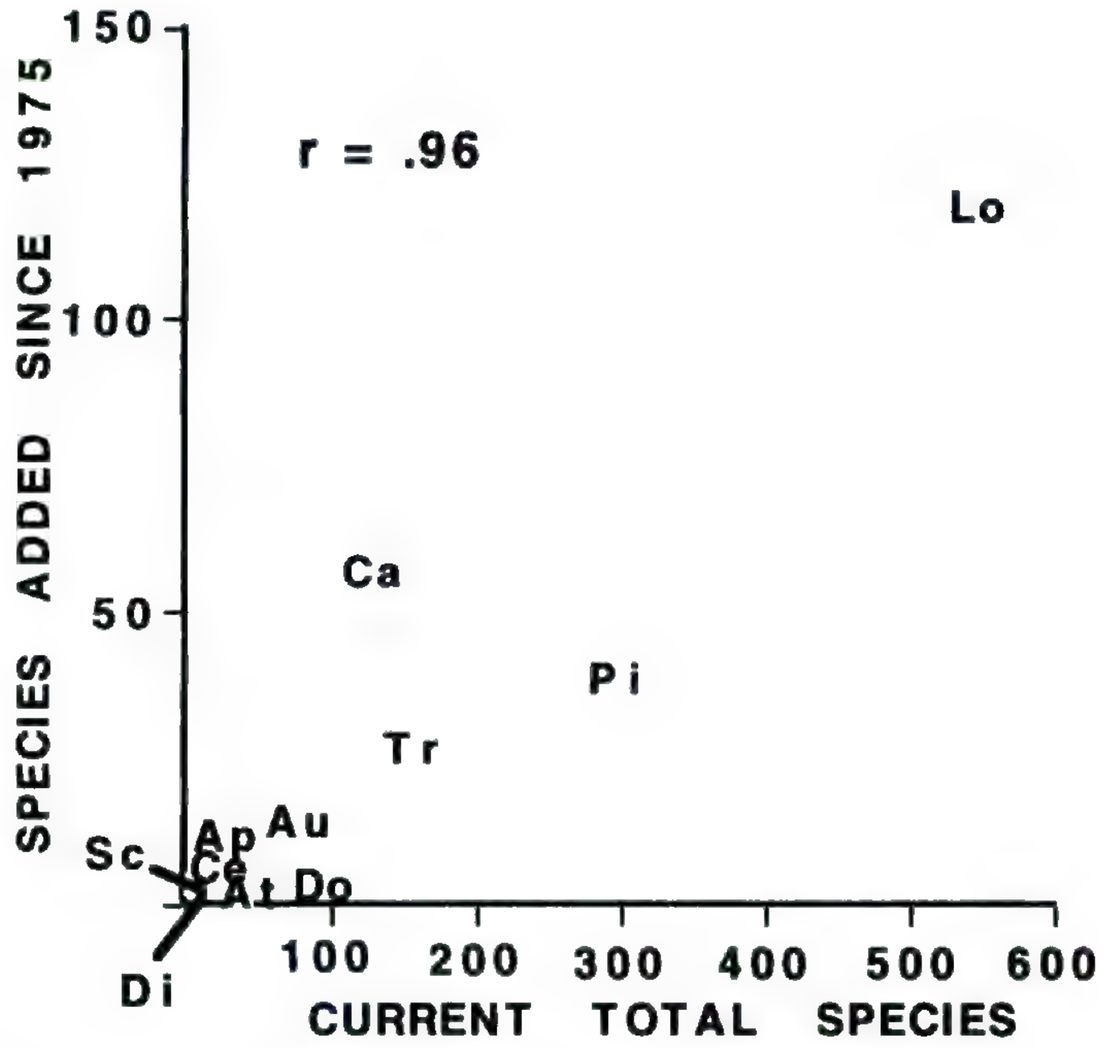


Figure 8. Relationship between species richness of Neotropical catfish families and numbers of new species described during the last ca. 25 yr. based on data from Eschmeyer (1998). Ap = Aspredinidae, At = Astroblepidae, Au = Auchenipteridae (including Ageneiosidae and Centromochlidae), Ca = Callichthyidae, Ce = Cetopsidae, Di = Diplomystidae, Do = Doradidae, Lo = Loricariidae, Pi = Pimelodidae, Sc = Scoloplacidae, Tr = Trichomycteridae. (One fossil but no extant species of Nematogenyidae were described in this period.)

for example, reported that by 1977, 31 nominal species of the cichlid genus *Apistogramma* had been described, of which he judged only 20 to be valid. In his 1980 revision, Kullander added 18 new *Apistogramma*; he recognized 36 overall and estimated that the genus contains > 40 species. By 1997 an additional 17 species of *Apistogramma* had been described, about a 50% increase in the 17 years following the initial revision. On the other hand, revisionary work may sometimes suggest conspecificity that might yield a net reduction in the number of recognized forms. In a revision of the widely distributed catfish genus *Rhamdia,* with about 100 nominal species, Silfvergrip (1996) proposed that only 11 species are valid (the author described 3 of these in the work).

Whereas the numbers of new species are high and the common taxa are becoming more so, the qualities of recent ichthyological discoveries in the Neotropics are perhaps more impressive than quantity. Neotropical fishes are famous for their diversity of diet and trophic apparati. New species with strikingly distinct feeding specializations are common. Among characiforms the most unusual recently found dietary specialist is the serrasalmine *Ossubtus xinguense* (Fig. 9A; Jégu, 1992), a species from turbulent cataracts that feeds on aquatic macrophytes, especially *Potemogeton.* During its devel-

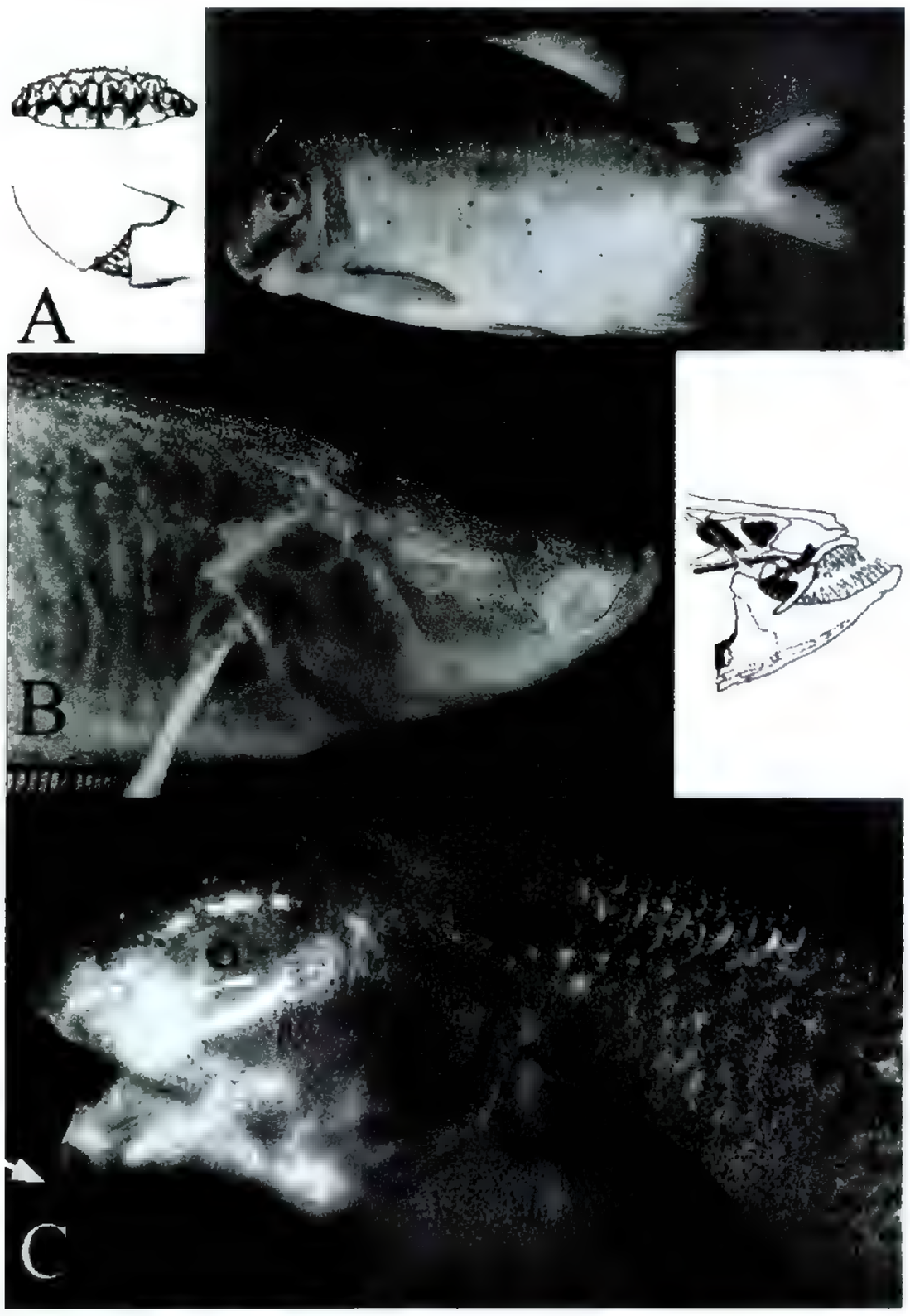


Figure 9. New dietary specialists from the Neotropics. —A. *Ossubtus xinguense*, left, drawings of teeth and mouth; right, an adult specimen about 153 mm long (reproduced with permission from Jegú, 1992). —B. *Magosternarchus duccis*, left, anterior third of adult specimen about 235 mm long; right, skeleton of teeth and jaws (reproduced with permission from Lundberg et al., 1996). —C. *Sternarchogiton* sp. (Copyright John G. Lundberg and John P. Sullivan.)

opment the snout, jaws, and gill chamber of *Ossubtus* undergo a metamorphosis, twisting ventrally so that the mouth and blade-like teeth are directed at the plants attached to the rocky substrate. An interesting twist was added to this case with the discovery of a parasitic isopod living in the gill chamber of the fish that also develops a contorted morphology, presumably in response to its host's cursive ontogeny (Thatcher, 1995).

Most gymnotiforms have generalized diets of small invertebrates or fishes. However, the recently described genus *Magosternarchus* (Fig. 9B; Lundberg et al., 1996) includes two species of Amazon Basin apteronotids that, with greatly enlarged jaws and teeth, feed on the tails of other electric fishes. Another "new" specialist among electric knife fishes is *Rhabdolichops zareti* (Lundberg & Mago-Leccia, 1986), from deep channels of the Orinoco, that feeds mostly on allochthonous zooplankton washed into the river's mainstem channels from the productive marginal savannas (Lundberg et al., 1987). Providing an example of an odd newly discovered structure possibly related to electrolocation of prey, an undescribed species of the apteronotid *Sternarchogiton* bears a probe-like, somewhat protrusible organ on its lower jaw (Fig. 9C).

The evolution of small to miniature-size species is commonplace among Neotropical fishes (Weitzman & Vari, 1988). It is no surprise that very small fishes are discovered relatively late in survey work; 40 of the 85 little species listed by Weitzman and Vari were described in the latest 25-year period, more than twice that of the previous quarter century, and several more have been discovered recently that fall below or approach their size criterion of 26 mm. In the Neotropics the evolution of reduced size is most common among characiforms, siluriforms, and cyprinodontiforms, and there are a handful of remarkably small engraulids (*Amazonsprattus scintilla*, Roberts, 1984) and gobioids (*Microphylipnus* species, Myers, 1927). Weitzman and Vari (1988) pointed out that miniature species of most taxa live in slow-flowing or still waters. The most strikingly modified small and miniature catfishes, however, are found in swiftly flowing and often deep, river channels. Examples of these include the aspredinid *Micromyzon akamai* (Fig. 10A; Friel & Lundberg, 1996), a blind, nearly pigmentless, heavily armored species that matures at about 12 mm, making it one of the smallest known catfish species. *Bathycetopsis oliveirai* (Fig. 10B; Lundberg & Rapp Py-Daniel, 1994) is a deep-water ca. 35-mm cetopsid catfish that has completely lost its eyes and pigment but has enormously hypertrophied olfactory organs. There are two new groups of very small, microphthalmic, channel-dwelling pimelodids: *Horiomyzon retropinnatus* (Fig. 10C; Stewart, 1986), with wing-like pectoral fins, and an undescribed clade with at least four species bearing thickened bones and skin studded with sensory organs.

At the other end of the size spectrum, there are occasional discoveries of large to very large fish species. Toledo-Piza (1997; Toledo-Piza et al., in press) has determined that the well-known fang-toothed cynodontine *Hydrolycus*, long considered monotypic, contains at least four species of which two are undescribed. Lundberg and Akama (1999, and in prep.) have identified an unrecognized species of "goliath" catfish, *Brachyplatystoma* (Fig. 10D), in the central and lower Amazon. This important species is now known to reach close to a meter in length, and it is among the most common in the commercial and artesanal fisheries where it is confused with *B. filamentosum*.

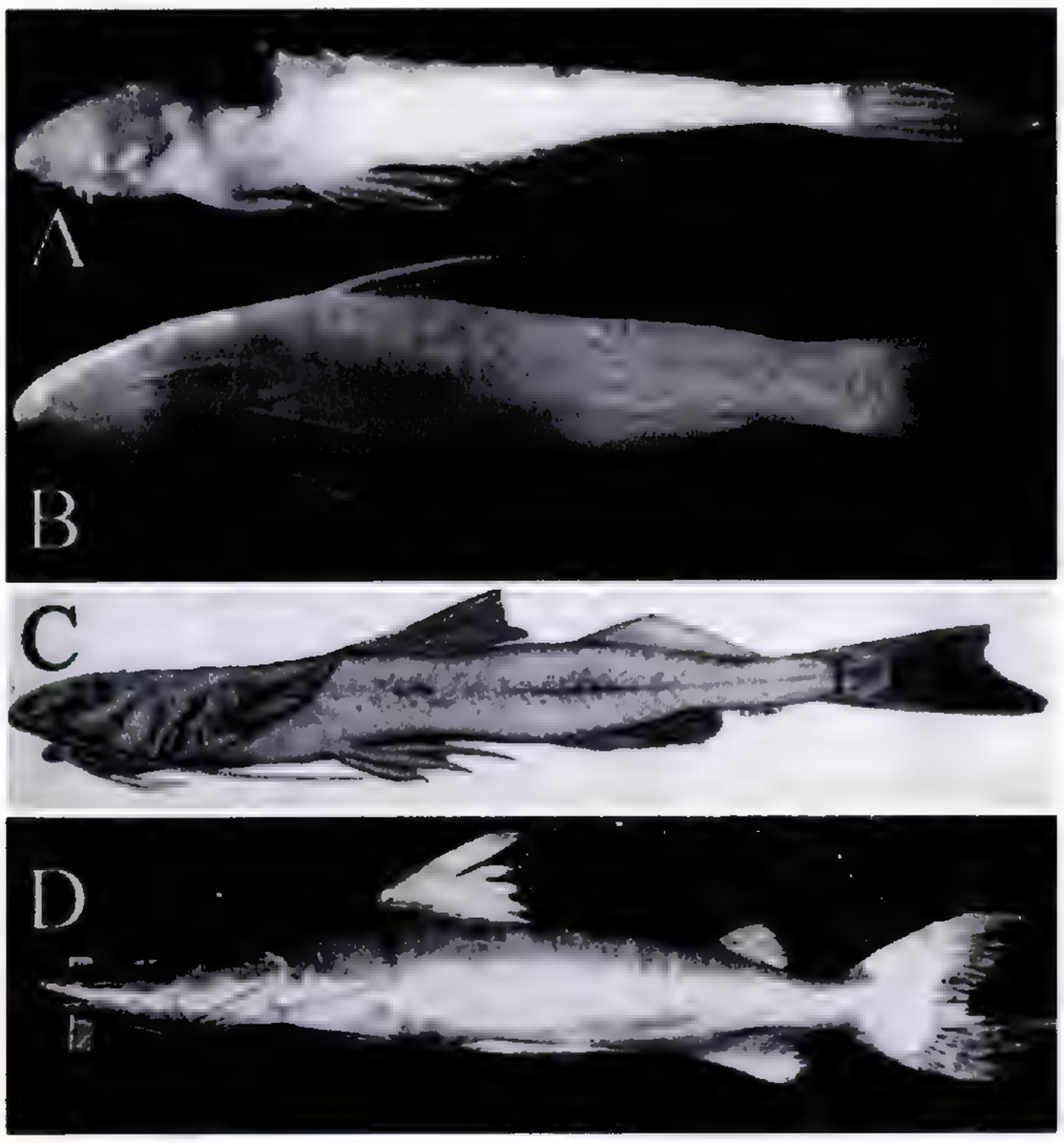


Figure 10. Recent discoveries of very small and large fishes in the Amazon. —A. *Micromyzon akamai*, 14 mm long (reproduced with permission from Friel & Lundberg, 1996). —B. *Bathycetopsis oliveirai*, 30 mm long (reproduced with permission from Lundberg & Rapp Py-Daniel, 1994). —C. *Horiomyzon retropinnatus*, 23 mm long (reproduced with permission from Stewart, 1986). —D. *Brachyplatystoma* sp., specimen about 500 mm long. (Copyright John G. Lundberg.)

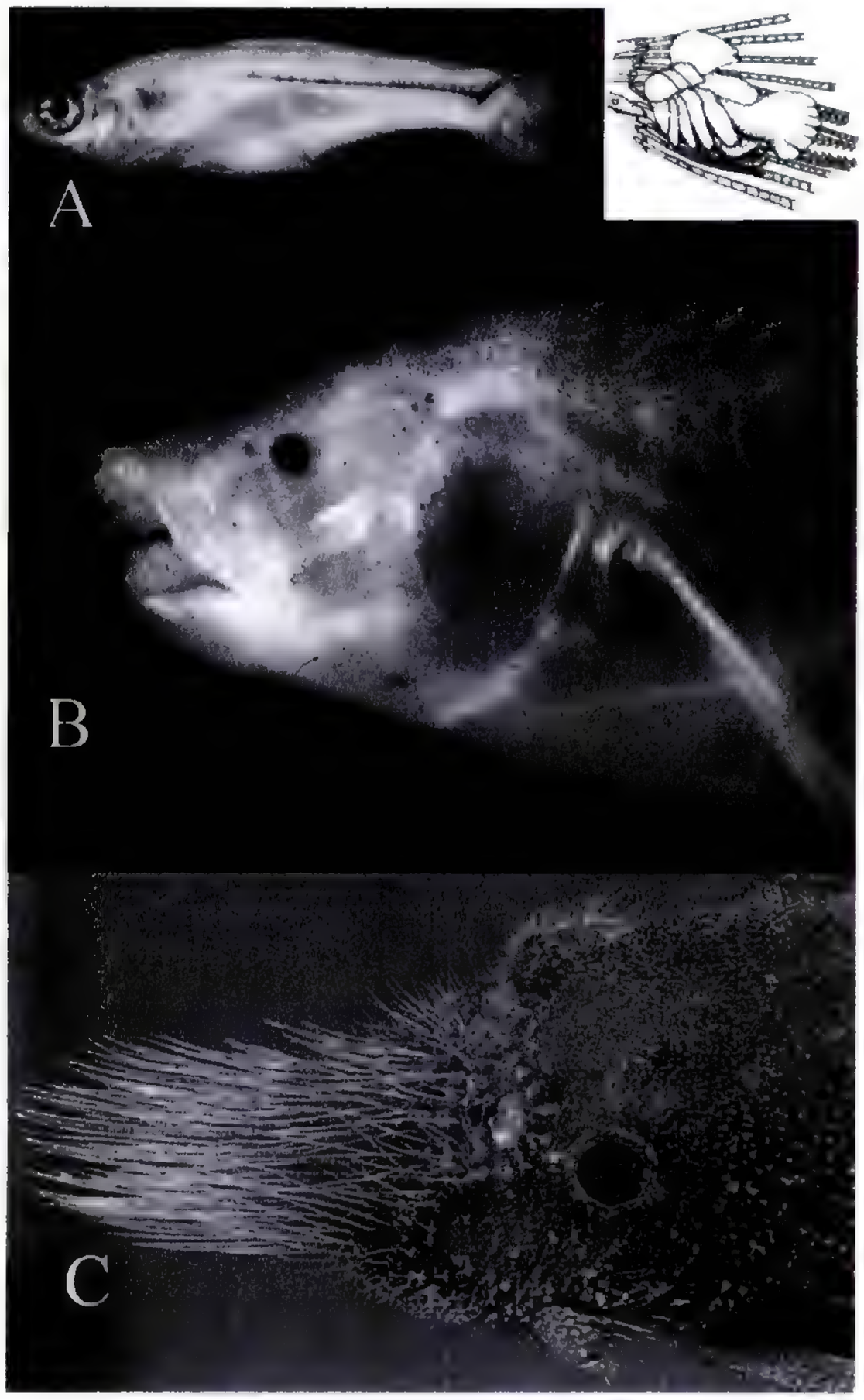


Figure 11. Extraordinary sexual dimorphism found in Neotropical fishes. —A. Left, *Xenurobrycon macropus*, male glandulocaudine characin, 15.4 mm long, with a caudal gland; right, modified scales surrounding caudal gland in male *Mimagoniates microlepis* (from Weitzman & Fink, 1985; permission to reproduce images granted by Smithsonian Institution, NMNH, Division of Fishes). —B. The nominal *Oedemognathus exodon*. (Copyright John G. Lundberg and John P. Sullivan). —C. *Neblinichthys pilosus*, male about 50 mm long (reproduced with permission from Ferraris et al., 1986).

Novel aspects of reproductive biology are being discovered in Neotropical fishes. In addition to the continuing discovery of new genera and species (see Weitzman & Menezes, 1998; Malabarba, 1998), recent investigations of the glandulocaudine and cheirodontine Characidae have turned up a new mode of reproduction for characiforms. These fishes are convergently sexually dimorphic, with males developing a variety of specialized, courtship-related features of the caudal fins such as bent fin rays with hooks and fleshy tissues that appear to be glandular (Fig. 11A; Burns et al., 1995, 1997; Malabarba & Weitzman, 1999). Almost all male glandulocaudines develop a cutaneous caudal gland thought to produce a pheromone, and the gland is surrounded by hypertrophied scales and in some species putative "pumping" muscles. Oddly, although the males lack any obvious intromittent organ, many of these species are internal fertilizers.

The known limits of sexually dimorphic characteristics are being stretched by some Neotropical fishes. Recent collections made during the high-water season near Manaus have turned up breeding associations of electric fishes and morphologically intermediate individuals showing two cases of extreme sex dimorphism that have misled taxonomic decisions. The nominal species *Aperonotus anas* is based on a large, hypermorphically long-snouted male of *A. hasemani* (Cox Fernandes, 1998; Cox Fernandes & Lundberg, 1999). The nominal, monotypic *Oedemognathus exodon* is based on large males of *Sternarchogiton nattereri* that develop strong externalized teeth, perhaps for use in combat over mates (Fig. 11B; Cox Fernandes & Lundberg, in prep.). Ferraris et al. (1986) described a bizarre loricariid armored catfish, *Neblinichthys pilosus* (Fig. 11C), in which males develop on their snouts a cartoon-like bush of elongate odontodes, i.e., cutaneous teeth.

Among discoveries during the last 25 years, the greatest scientific interest attaches to previously unseen fishes that represent new phylogenetic lines

at or above the family level. These discoveries have been most common among catfishes and often result in broad changes in higher classification. The loricarioid *Scoloplax dicra* (Fig. 12A, B; Bailey & Baskin, 1976) was first described as a new subfamily of Loricariidae, but was soon elevated to family rank by Isbrücker (1980). Three more *Scoloplax* species were described by Schaefer et al. (1989), and Schaefer (1990) determined that this clade is the likely sister taxon of the vast assemblage of Loricariidae + Astroblepidae.

The Trichomycteridae presently include about 200 species (perhaps over 100 in the large genus *Trichomycterus*) arranged in eight or nine subfamilies (de Pinna, 1998). The most widely known trichomycterids are the "candirús" or "parasitic catfishes" that feed within the gill chambers of larger fishes on blood and epithelia. (Candirús finally earned their notoriety for supposed inadvertent entry into the urethrae of humans with the first medically verified case of such behavior in Brazil in 1998.) Of more scientific interest, however, is the recent explosion of systematic knowledge of the perplexing diversity of trichomycterids. Although 16 of the 27 trichomycterid species described between 1975 and 1997 belong to *Trichomycterus*, the remaining 11 (40%) are placed in new or previously rare, poorly known genera and subfamilies. The highly derived *Trichogenys* (Fig. 12C, D; Britski & Ortega, 1983) has been placed in its own subfamily by Isbrücker (1986). Two newly described genera, *Copionodon* (Fig. 12E, F) and *Glaphyropoma*, form a small, plesiomorphic clade of three species that de Pinna (1992) placed as the sister taxon to all other trichomycterids. The subfamilies Sarcoglanidinae and Glanapteryginae, known from very few specimens, contain perhaps the most extraordinarily specialized and diminutive catfishes in the world (Fig. 12G, H). In addition to major range extensions, in just the last decade the ranks of the sarcoglanidines were increased by four new monotypic genera (e.g., Fig. 12K, L; de Pinna, 1989; de Pinna & Starnes, 1990; Costa & Bockmann, 1994b; Costa, 1994), and two species and one genus were added to the glanapterygines (de Pinna, 1988, 1989; Costa & Bockmann, 1994a). It is no surprise that this remarkable amplification of trichomycterid diversity at the genus and family-group levels has caused a significant revision of our phylogenetic understanding (de Pinna, 1992, 1998; Stiassny & de Pinna, 1994). This case emphasizes the major impact that taxon sampling can have in Neotropical fish systematics (see also Schaefer, 1998).

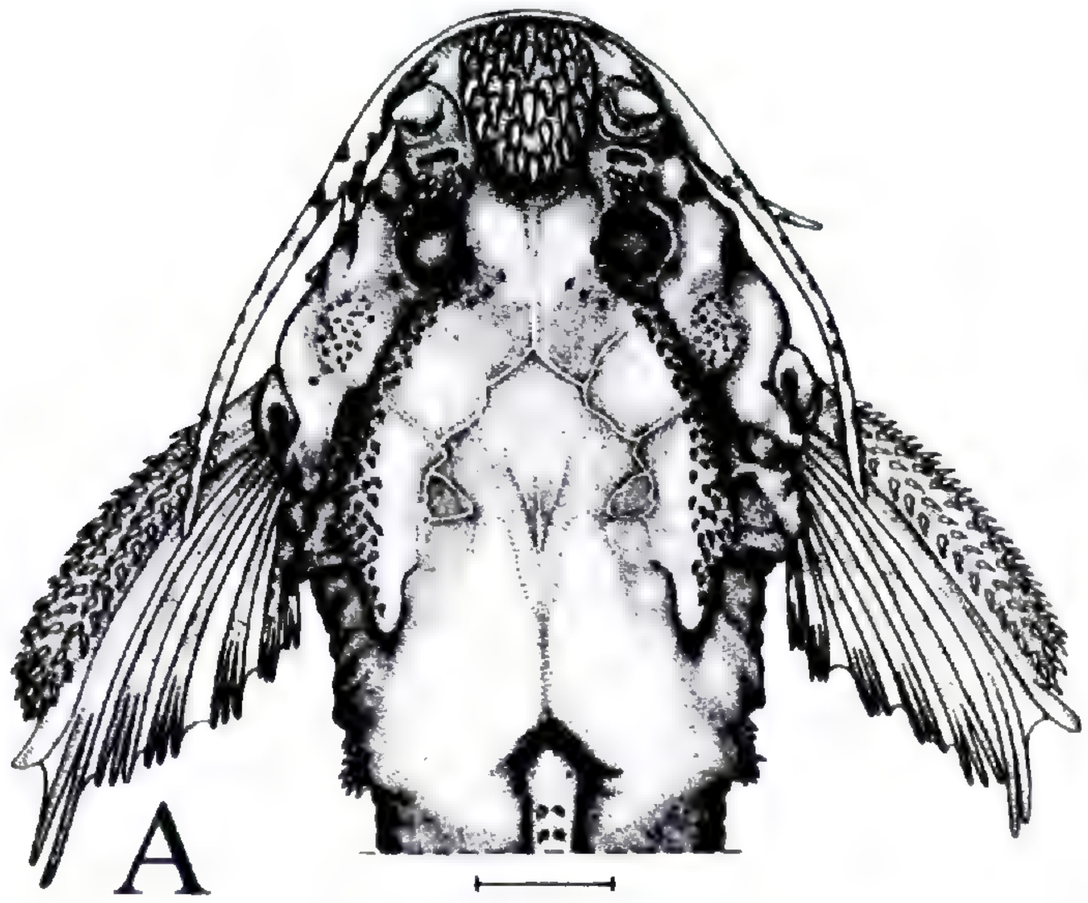
Discoveries of new species and new character evidence in known clades often lead to altered phylogenetic arrangements and classifications. Examples include the catfishes *Helogenes*, discovered to be closely related to the Cetopsidae (de Pinna & Vari, 1995), and *Hypophthalmus* (Fig. 13A), which is now confidently placed within Pimelodinae instead of in isolation in its own family (Howes, 1983; Lundberg et al., 1991). The first examinations (only within the last three years) of skeletal specimens of the monotypic, rio São Francisco endemic *Conorhynchos conirostris* (Fig. 13B) have revealed that this supposed pimelodid, described in 1840, does not belong to any diagnosed family group of siluriforms (Bockman, de Pinna & Ferraris, pers. comm., pers. obs.). There are noteworthy recent higher-level discoveries in other Neotropical groups as well. Stiassny (1991) has found synapomorphies that unite New World cichlids as the monophyletic sister taxon of some African cichlids. On the other hand, there is increasing evidence of multiple sister-taxon relationships between African and South American characiform and cyprinodontiform clades (Parenti, 1981; Vari, 1995; Buckup, 1998; Costa, 1998).

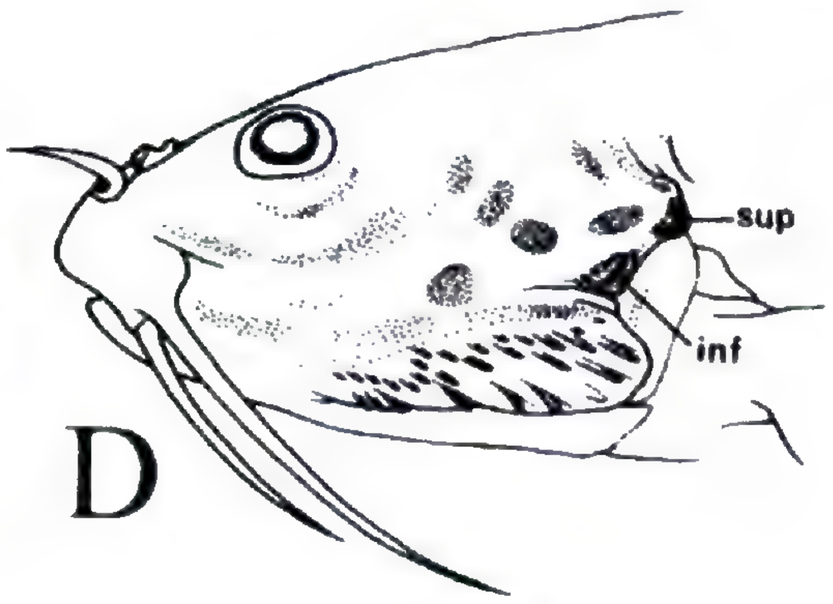
Thus, at every level knowledge of Neotropical fishes continues to grow at remarkable rates: myriad species, their intra- and intercontinental relationships, and their richness and novelty of characteristics and natural history. Prospects for ichthyological discovery in South and Central America continue to be as high as ever.

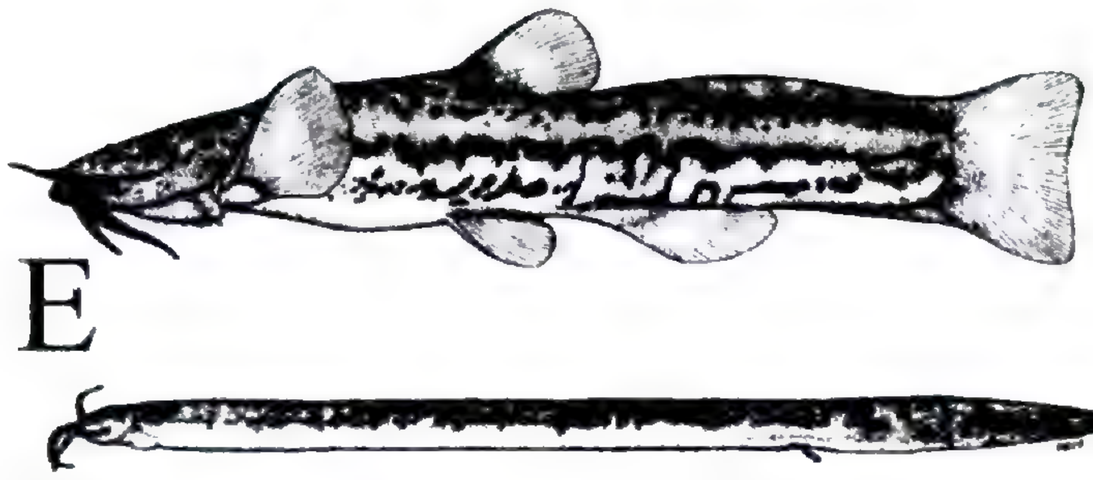
EUROPE

Europe west of the Ural Mountains comprises roughly one-third of the Palearctic Realm (Fig. 2). The major groups accounting for most (ca. 80%) of the fish species are Cyprinidae (129), Salmonidae (54), Coregonidae (43), Gobiidae (31), Cobitidae (21), Petromyzontidae (11), Clupeidae (11), and Percidae (11). The "classical" checklists of European freshwater fishes are outdated and reflect a state of knowledge and species concepts of 30–40 years ago. Maitland (1976) listed 215 species for Europe west of the Urals and 170 species in Europe exclusive of the former U.S.S.R. In a recent review Kottelat (1997) recognized 358 species in Europe (excluding the former U.S.S.R.), an increase of 111%.

Northern Europe, much of it glaciated during the Pleistocene, has low fish diversity, usually with only 2 or 3 native species in any watershed. The number of species increases to the south. North of the Alps from France to Russia, the fauna of about 30 species is relatively uniform. The Danube basin, with







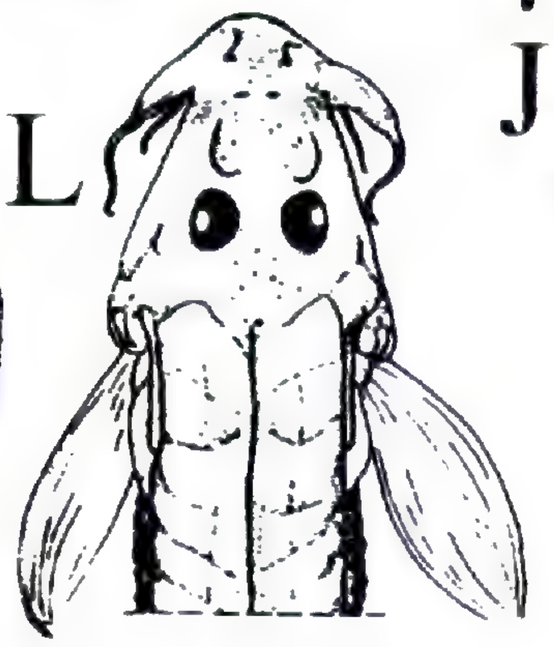


Figure 12. Recent discoveries of phylogenetic importance among South American scoloplacid and trichomycterid catfishes. —A, B. The scoloplacid *Scoloplax dicra*, scale bars 1 mm (reproduced with permission from Bailey & Baskin, 1976). —C, D. The trichogenine *Trichogenys longipinnis* (from Britski & Ortega, 1983). —E, F. The copionodontine *Copionodon pecten*, about 60 mm long (reproduced with permission from de Pinna, 1992). —G, H. The glanapterygine *Glanapteryx anguilla*, about 55 mm long (reproduced with permission from de Pinna, 1989). —I, J. The glanapterygine *Listrura camposi*, about 38 mm long (reproduced with permission from de Pinna, 1988). —K, L. The sarcoglanidine *Stauroglanis gouldingi*, about 23 mm long (reproduced with permission from de Pinna, 1989).

Figure 13. Recent discoveries of phylogenetic importance among long-known South American catfishes. —A. *Hypophthalmus fimbriatus.* —B. *Conorhynchos conirostris.* (Copyrights John G. Lundberg and John P. Sullivan.)

Figure 14. Valid European cyprinid species previously hidden by synonymy. —A. *Scardinius acarnanicus,* 152 mm long. —B. *Scardinius graecus,* 140 mm long. (Copyrights Maurice Kottelat.)

about 90 species, has the most diverse fauna on the continent. The fish faunas of the southern European peninsulas contain relatively high numbers of endemic species but are otherwise species-poor assemblages: 87 species in the Balkan Peninsula, 29 in the river basins of the Italian Peninsula, and 26 in the Iberian Peninsula. The only endemic European fish family is the cyprinodontiform Valenciidae, with two species. This fauna has its closest relationships with Asia, North America, and northernmost Africa.

The cornerstones of European fish systematics were provided in the 16th century in Rondelet's *Libri de piscibus* (1554) and Gesner's *Nomenclator aquatilium* (1560) and *Fischbuch* (1563). Modern European ichthyology started with Peter Artedi's *Ichthyologia* (1738), whose system and nomenclature were adopted by Carl von Linné in 1758.

Having thus enjoyed 450 years of study (about double that of any other continental fish fauna), one would expect that our modern systematic knowledge of European fishes is definitive. This is not the case, and even the most basic question, "How many species?," has no easy answer. That approximately 2000 names have been applied to European fish species suggests that their taxonomy is not simple (Kottelat, 1997). As with North America, the discovery of previously unknown fish species in European waters is now uncommon. How then do we account for great increase in the number of valid European fish species proposed by Kottelat (1997)?

First, there have been some recent discoveries of a few previously unknown species. Twenty species were discovered in European waters between 1978 and 1998, and some 10 known species are still unnamed. These are cyprinids, cobitids, and gobiids of small to very small size (3 to 10 cm). Most were found in southern Europe on the Iberian, Italian, and Balkan peninsulas. In 1998, two new cyprinids and one new cobitid were described from the Iberian Peninsula.

Second, several species described by earlier authors and considered invalid by later authors, have since been demonstrated to be distinct. This also mainly concerns species from southern Europe. For example, the species of the cyprinid genus *Scardinius* have all been treated as synonyms of *S. erythrophthalmus.* It appears that at least five species should be recognized, some of which are conspicuously distinct from *S. erythrophthalmus* (Fig. 14) (Iliadou et al., 1996; Kottelat, 1997: 82).

Another interesting case is the goldfish *Carassius gibelio,* originally described from Germany in 1782. This species is traditionally considered as either the wild form of the goldfish, a feral goldfish, or a hybrid. These theories ignore the Japanese literature on morphology and karyotypes of goldfishes that documents the existence of at least five species of *Carassius* in east Asia, all of them distinct from *C. gibelio* (see Hosoya, in Nakabo, 1994, for a summary). They ignore the fact that *C. gibelio* was apparently recorded in European waters as early as 1549 (Kentmann's Codex; Hertel, 1978), long before goldfish were first imported to Europe in 1611 or 1691, and maybe even before it was imported from China to Japan (between 1502 and 1748). The hybrid theory does not seem to have support. So given our current knowledge, there is no alternative but to recognize *Carassius gibelio* as a distinct species.

Third, application of the Phylogenetic Species

Concept (PSC) has played a role in increasing the number of recognized species (Kottelat, 1997). In this view, a species is "an irreducible (basal) cluster of organisms diagnosably distinct from other such clusters, and within which there is a parental pattern of ancestry and descent" (Cracraft, 1989: 34–35). Most earlier work on European fishes either used some sort of implicit Biological Species Concept or, more frequently, was not explicit regarding concepts, definitions, and criteria. Inconsistencies in application of criteria are common. Detailed studies of geographic variation and systematics reveal that fish diversity often has been underestimated, and there is no theoretical or practical advantage in underestimating biodiversity. Subspecies of earlier authors that satisfy species criteria under the PSC must be treated as species. Further, some traditionally recognized subspecies have been found to be nothing more than arbitrarily delimited sections of clines, and these entities must be abandoned because there is no sense in recognizing arbitrarily defined units.

The systematics of salmonids and coregonids has been notoriously difficult because of real or perceived high degrees of character plasticity and failure to find consistent morphological differences among populations. Most earlier treatments avoided dealing critically with the systematics of salmonids and coregonids, and this resulted in a much underestimated number of species. Some "classical" lists merely include a single coregonid taxon, "*Coregonus* sp." The species-level systematics of these families is still far from resolved, but a pragmatic handling of their taxonomy is possible. Using a few simple concepts and definitions, Kottelat (1997) tentatively recognized 44 monophyletic evolutionary units within the genus *Coregonus* in Europe that can be recognized as species (cf. 7 species recognized by Blanc et al., 1971), and 27 within the genus *Salmo* (cf. 5 recognized by Stearley & Smith, 1993). Careful comparisons often show constant differences in morphology and in color patterns, and this is now supported by molecular data (e.g., Bernatchez & Dodson, 1994; Bernatchez et al., 1992). The distinctive endemic species of *Salmo* provide examples (Fig. 15). Among these, *S. trutta* (Rhine basin) and *S. rhodanensis* (Rhône basin) occur in creeks only a few kilometers apart along the divide between the two basins in western Switzerland. These species have always been empirically treated as different on the basis of coloration and morphology, a fact corroborated by molecular data.

Some may object that it will be impossible to handle the large number of names that application of the PSC will produce. This is, however, not the

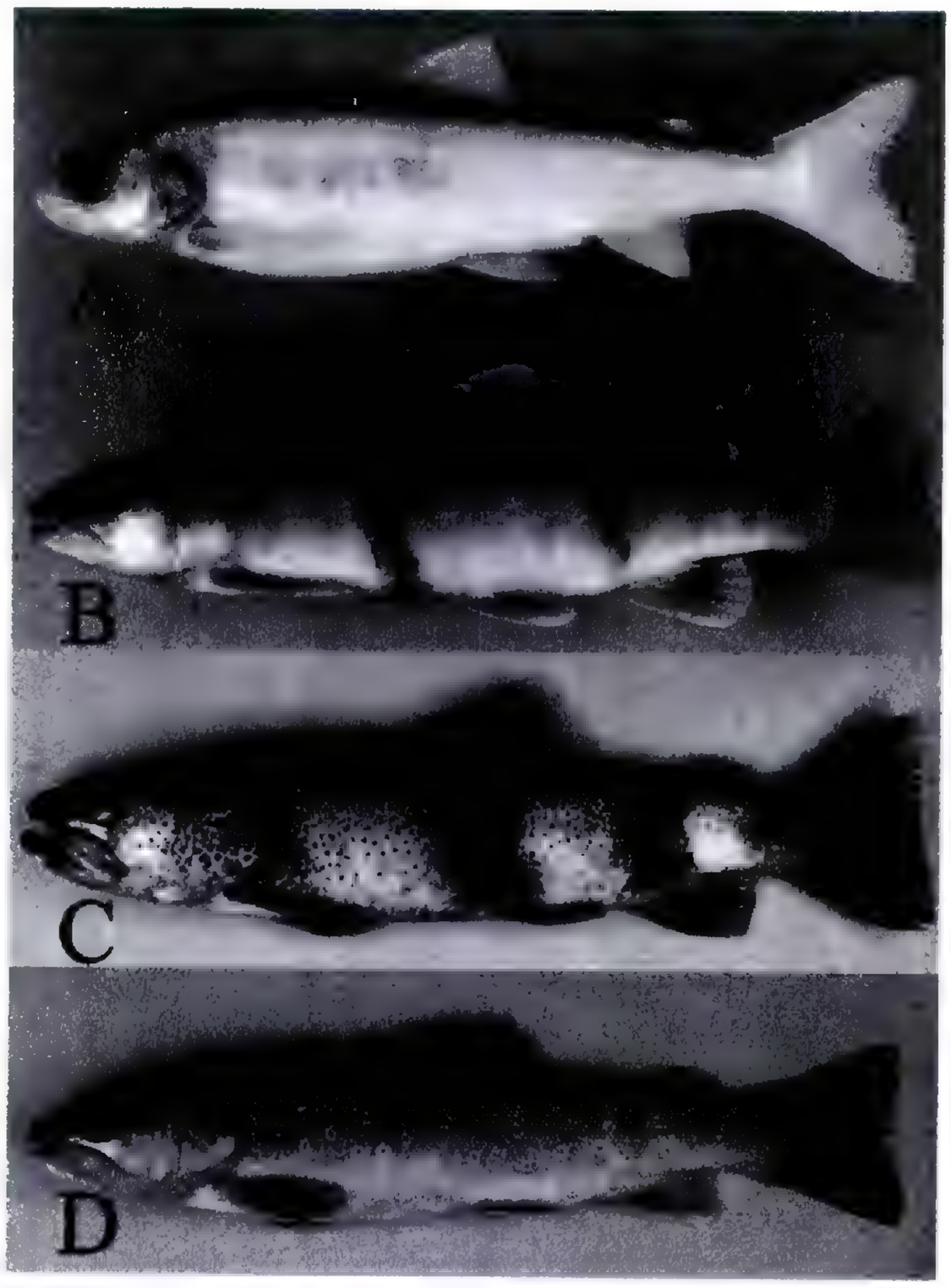


Figure 15. Examples of three endemic and one wide-ranging species of European *Salmo*. —A. *Salmo carpio*, juvenile, 133 mm long, endemic to Lake Garda, Italy. —B. *Salmo peristerus*, 236 mm long, endemic to the Lake Prespa basin, Greece-Albania-FYROM. —C. *Salmo rhodanensis*, 305 mm long, endemic to the Rhône basin. —D. *Salmo trutta*, 235 mm long, specimen from Rhine basin, Switzerland, caught about 5 km from C. (Copyrights Maurice Kottelat.)

point; if they are different species we have to handle them as species, give them names as species, and manage them as species. The problem is not the names, but with our perceived limitations. Artificially squeezing all trout species into the same pigeonhole risks confusing critical information about species distinctions. If details of ecology of one species are mixed with the migration data of another and the reproduction of a third one under a single name, it is not surprising that patterns of species diversity are blurred.

Salmonoids are notorious also for the existence in many lakes of several sympatric stocks that have been classically considered as different "forms" of a single species. In many cases, the "forms" are not only morphologically different, but have different habitats, prey on different organisms, have different spawning seasons and spawning grounds, and are genetically distinct (but few have been investigated on this last aspect). Under any species concept, they are different species, and several of these "polymorphic species" thus are groups of

species or possibly species flocks. Presently, the best example is probably the three or four species of *Salvelinus* of Lake Thingvalla in Iceland. European examples are the *Salmo* of Lough Melvin (Ireland) and Lake Ohrid (Albania and the former Yugoslavian Republic of Macedonia) and the *Coregonus* of Lake Konstanz and Lake Thun.

AFRICA

According to current estimates, Africa (Fig. 2) harbors some 2850 species arrayed, depending upon taxonomic convention, within 40–50 families. While perhaps not as rich in species as other tropical regions, what Africa lacks in terms of raw numbers it makes up for in an ichthyofauna that includes some striking examples of evolutionary phenomena ranging from relictual "living fossils" and strange morphological isolates, to stunningly diverse species flocks exhibiting unparalleled speciation rates and adaptive radiations. It is noteworthy that African fish diversity is marked by an interesting preponderance of archaic, phylogenetically isolated taxa. Fishes such as bichirs (Polypteridae), lungfishes (Protopteridae), the butterflyfish (Pantodontidae), weakly electric elephantfishes (Mormyridae), the denticle herring (Denticipitidae), the snake mudhead (Phractolaemidae), and knerids (Kneriidae) are among the many examples found today only in African freshwaters. Perhaps more familiar, and certainly more numerous, are members of the two major freshwater fish radiations: the otophysan cyprinids, characiforms, and siluriforms and the percomorph cichlids. In Africa an interesting taxonomic dichotomy between riverine and lacustrine communities exists. The former are dominated by otophysans: cyprinids, characiforms, and a few dominant catfish families (Clariidae, Mochokidae, and Claroteidae), along with mormyrids and cyprinodontiforms (particularly in rivers of west and central Africa). On the other hand, Africa's many lacustrine ecosystems, particularly those of the Great Rift Valley, are almost completely dominated by remarkable adaptive radiations of cichlid species.

Given the enormous size of the African continent, an ichthyological tally of 2850 species, although unquestionably an underestimate of actual species richness, is remarkably low. By way of partial explanation, the common vision of Africa's inland waters as characterized by the great Nile, Niger, Congo (Zaire), and Zambezi Rivers is misleading. In fact, over 90% of Africa's rivers are less than 9 km in length and many flow only seasonally. Surprisingly, Africa has more arid and semi-arid area than any other continent on the planet, and as a result the great majority of Africa's fish biodiversity is concentrated in the tropical, moister (and historically forested) regions.

Roberts (1975), building on foundations laid by Boulenger (1905) and Pellegrin (1911, 1921), divided Africa into 10 so-called ichthyofaunal provinces reflecting broad areas of endemicity. Greenwood (1983) has likened the state-of-the-art to bioaccountancy rather than biogeographic analysis, and Leveque (1997) noted recently that the biogeography of African freshwater fishes remains poorly known. The problems are manifold: new taxa are constantly being discovered, distribution patterns for most taxa are poorly known, and range extensions often encompassing thousands of kilometers are far from uncommon, but perhaps most importantly there is a profound lack of adequate hypotheses for the phylogenetic relationships of the species involved. Despite these shortcomings, the ichthyofaunal provinces as currently recognized (see Leveque, 1997, for an up-to-date summary) are a useful first-pass approach to subdividing the ichthyofauna into meaningful biogeographic units. Unquestionably, the fauna is phylogenetically richest and most varied in the rivers of tropical west and central Africa, with numbers attenuating in the provinces to the north and south of the tropical band. However, in terms of raw species numbers, it is the cichlids of the lakes of east Africa that dominate the continent's inland waters.

Overall African fishes exhibit a very high level of endemism; almost 100% at the species level and upward of 40% at the familial, no doubt a reflection of the long-term stability and isolation of the African continent. To the extent that extracontinental affinities are known, it seems that the dominant relationship is with Asia (e.g., Notopteridae, Cyprinidae, Bagridae, Schilbeidae, Clariidae, Channidae, Anabantidae, Mastacembelidae, Scatophagidae, pellonuline clupeids, and etropline cichlids), although links with South America (e.g., Lepidosirenidae, arapaimid osteoglossomorphs, some characiforms, siluriforms, cyprinodontiforms, and cichlids) and Europe (some cyprinid genera) are also evident in a few clades. Unfortunately, our current understanding of the phylogenetic relationships of most African clades is insufficient to enable historical interpretation of many distributional data.

While the first African fishes to reach European collections were assembled by the French naturalist Michel Adanson during his stay in Senegal (1749–1753), much early exploration in Africa was centered on the Nile, a river that has played a major

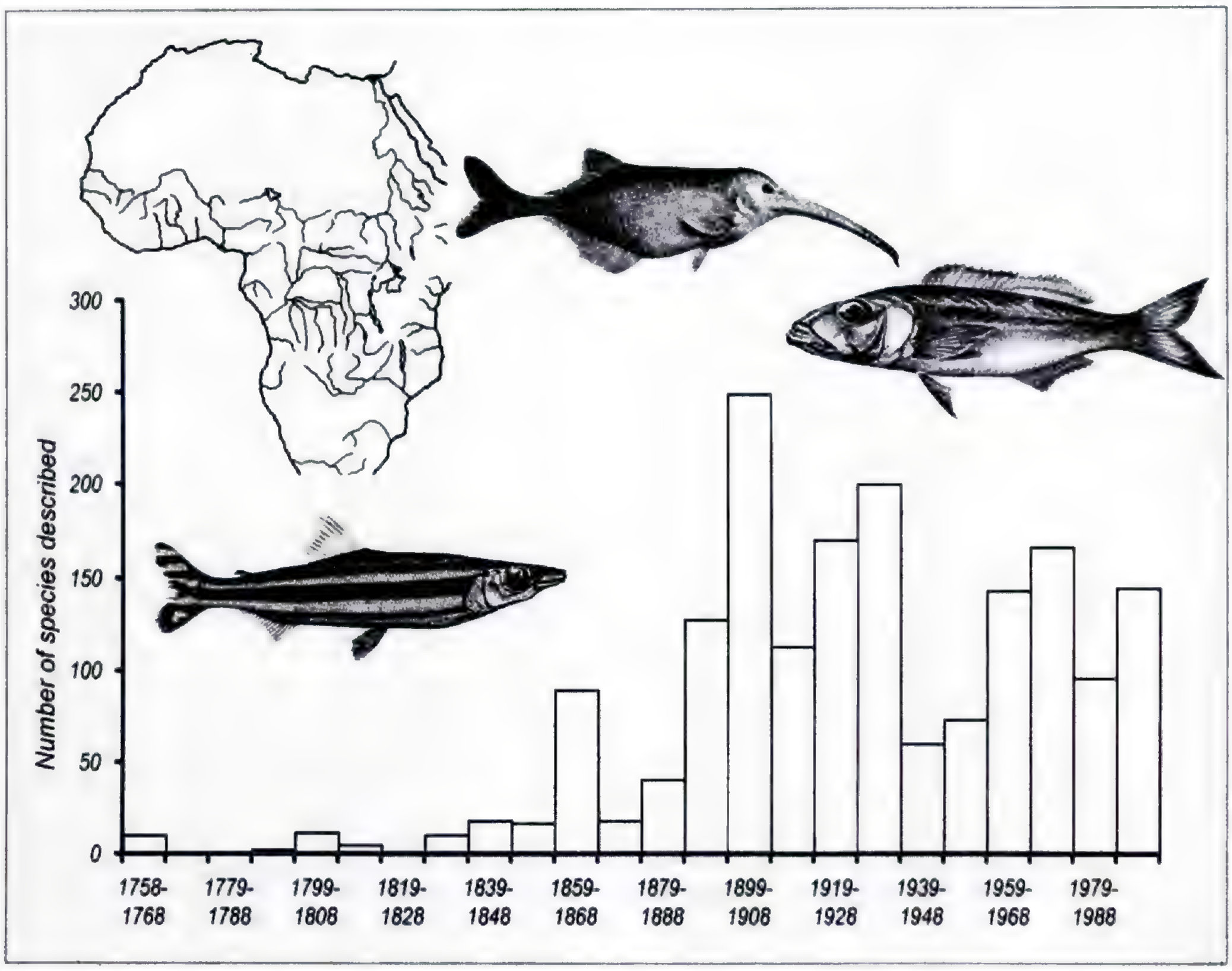


Figure 16. Species discovery plotted for three African fish clades (Citharinidae, Mormyridae, and Cichlidae). Black bars indicate the number of species described in 10-year increments from 1748 until 1998. Data compiled mainly from Eschmeyer (1998).

role in the history of Mediterranean civilization (see Beadle, 1974, for an excellent discussion of early exploration of Africa's inland waters). Daget (1994) recounted an amusing anecdote in which it is claimed that on Geoffroy Saint-Hilaire's return from the Napoleonic Campaigns in Egypt (1799) his fish collections were deposited at the Paris Museum. When Cuvier examined for the first time the extraordinary bichir (*Polypterus*), he is reputed to have declared, "This alone is justification for the Egyptian Campaign." Whatever the importance of nilotic fishes, the real beginnings of modern African ichthyology are perhaps best marked by the publication of Cuvier and Valenciennes' multi-volume *Histoire naturelle des poissons* (1828–1849), in which about 140 African fishes were described. Some 50 years later when Boulenger published his four-volume Catalog (1909–1916) he was able to record over 1400 species. And by 1991, with the publication of the last in an extremely useful series of checklists (Daget et al., 1984, 1986, 1991), the tally had risen to about 2850. Although Boulenger's Catalog remains the only pan-African faunal study, numerous regional accounts have since been published. Two in particular, Leveque et al. (1990, 1992) for most of west Africa and Skelton (1993) for southern Africa (including the Zambezi province), have comprehensive coverage. Additionally, much up-to-date regional information is to be found in Teugels et al. (1994), and references to major revisional studies are to be found in Leveque (1997). As was noted earlier, the great majority of African species are members of just two groups: the otophysan cyprinids (ca. 475 species in 23 genera), characiforms (characids and citharinids with ca. 208 species in 39 genera), and clariid (ca. 74 species in 12 genera), clarioteid (ca. 98 species in 18 genera), and mochokid (ca. 167 species in 10 genera) catfishes; and the percomorph cichlids (ca. 870 species in 143 genera). Together these seven families comprise more than two-thirds of the continent's ichthyofauna.

Despite almost 200 years of scientific study, there remains a remarkable amount to learn about African fishes, and many surprises no doubt remain. At the most basic level, monitoring species numbers (Fig. 16) illustrates the cumulative discovery rates for three typical African fish clades and, as elsewhere in tropical freshwaters, species discovery continues, sometimes at an extraordinary

Figure 17. Recent discovery of an unknown assemblage of rock-dwelling cichlids in Lake Victoria. (Reproduced with permission; copyright Ole Seehausen.)

rate. For example, recent sampling in east Africa's Lake Victoria has revealed the existence of a previously unknown assemblage of over 120 stenotopic rock-dwelling cichlid species (Fig. 17) (Seehausen, 1996; Seehausen et al., 1998). The quite unexpected discovery of this extraordinary radiation not only has major implications for our understanding of the evolutionary dynamics of Africa's cichlid flocks (Kaufman et al., 1997; Turner, 1997) and their conservation (Kaufman, 1992; Kaufman & Ochumba, 1993; Seehausen et al., 1997), but it also illustrates that even in supposedly well-sampled locales there still remain many unknowns.

Equally illustrative is a recent study of the Cross River drainage of Cameroon and Nigeria. Although the Cross was considered reasonably well sampled when Teugels et al. (1992) thoroughly reviewed recently collected and historical material in museums, they found that previous figures underestimated true diversity by more than 70% (Stiassny, 1996). Even after that comprehensive study new taxa are turning up. For example, Stiassny, Schliewen, and Freihof (in prep.) are describing an enigmatic new genus of cichlid fish recently collected in the Cross (Fig. 18). This taxon, which is highly distinctive in appearance, displays a fascinating combination of character states that render its phylogenetic placement problematical and may result in significant changes to current views of relationships among tilapiine cichlids (a major clade of African cichlids of considerable economic importance). Another recent discovery altering our view of the evolutionary dynamics of tilapiine cichlids is that of the Lake Bermin species flock (Fig. 19). Lake Bermin, a small Cameroonian crater lake of little more than 0.5 km^2 has recently been determined to be home to the continent's first species flock of substrate-spawning tilapiines. The flock in-

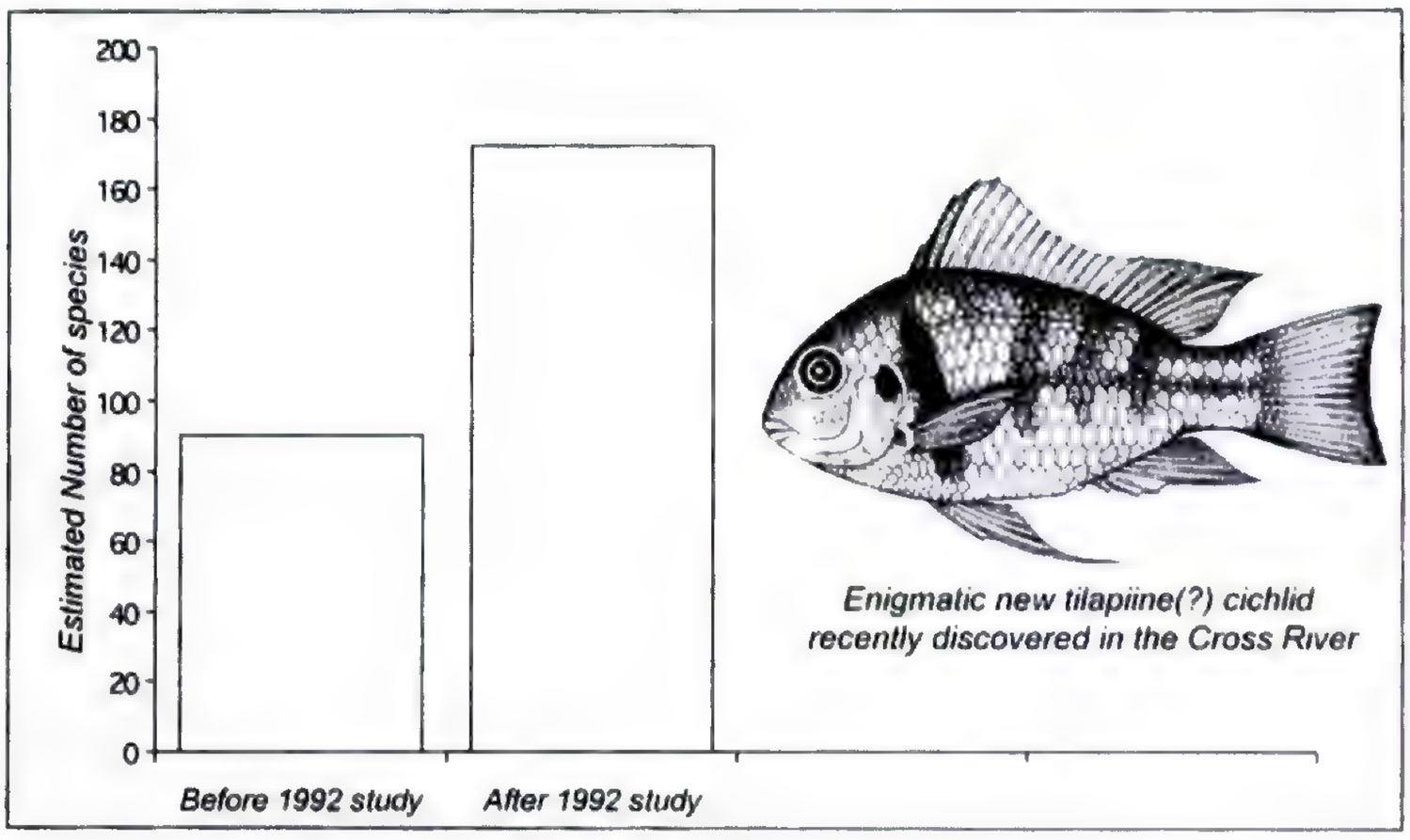


Figure 18. Burgeoning estimates of fish diversity in the Cross River basin of West Africa. Data compiled from Teugels et al. (1992).

cludes the smallest known tilapia, *Tilapia snyderae*, a species that attains sexual maturity at only about 25 mm. The discovery of endemic cichlid flocks in small crater lakes in the Cameroonian highlands provides a marvelous series of models for the study of evolutionary diversification and speciation mechanisms (Trewavas et al., 1972; Stiassny et al., 1992). For example, Schliewen et al. (1994) recognized the Lake Bermin and Lake Barombi Mbo cichlid flocks as providing the most compelling examples of probable sympatric speciation known among vertebrates.

The cichlid radiations of the east African lakes, likened to "living laboratories of evolution," provide some of the most extraordinary examples of vertebrate speciation, adaptive radiation, and ecomorphological diversification known on the planet and have intrigued biologists since their initial discovery at the end of the last century (e.g., Moore, 1903; Fryer & Iles, 1972; Fryer, 1976; Greenwood,

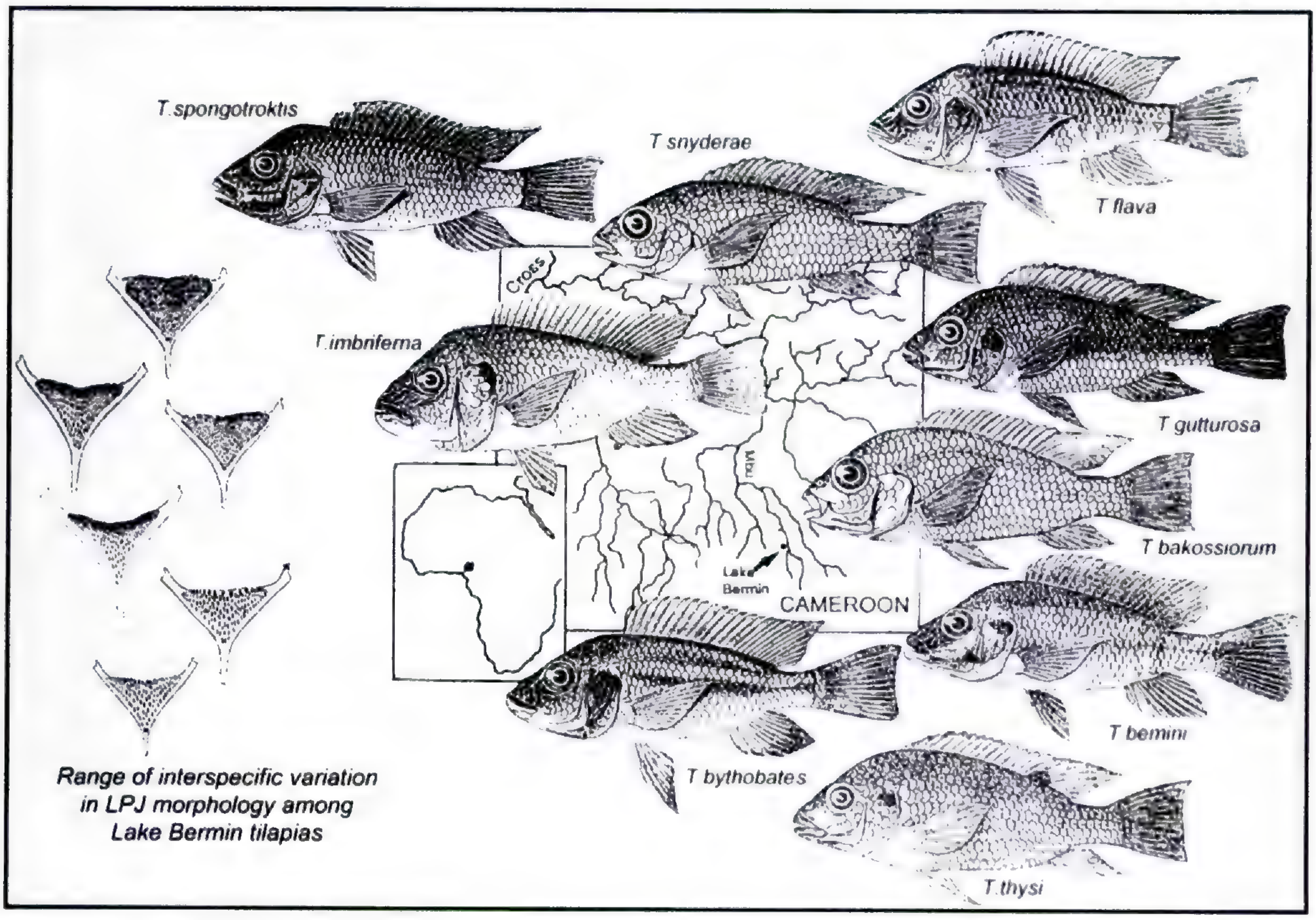


Figure 19. The Lake Bermin (Cameroon) *Tilapia* species flock. Data and illustrations after Stiassny et al. (1992).

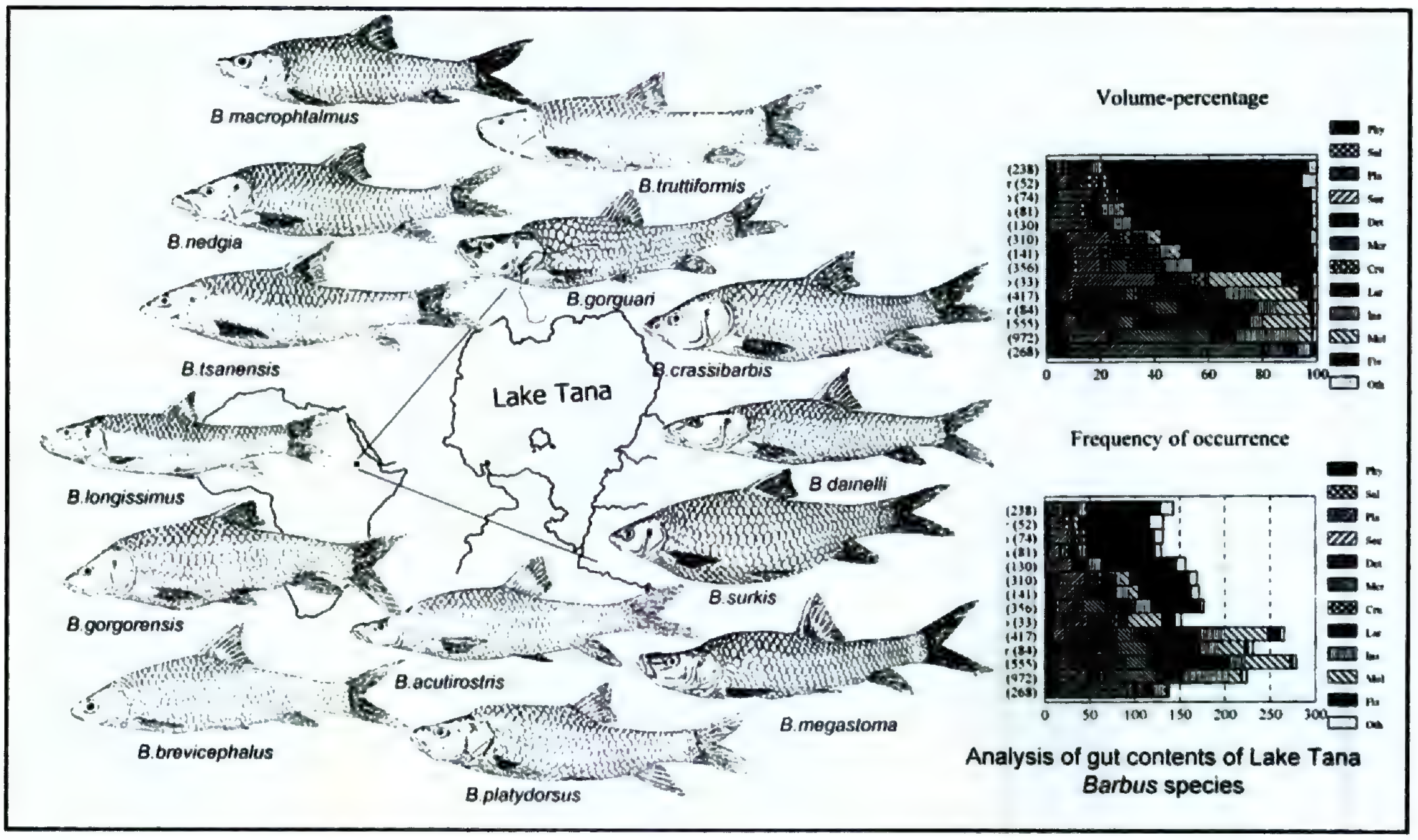


Figure 20. The Lake Tana (Ethiopia) *Barbus* species flock. Data and illustrations after Nagelkerke (1997).

1981; Coulter et al., 1986; Galis, 1998; Stiassny & Meyer, 1999). The recent explosion in molecular sequencing techniques and analytical methods has resulted in a plethora of studies aimed at unravelling phylogenetic, biogeographic, and speciation mechanisms among these fishes (e.g., Sturmbauer & Meyer, 1992; Meyer, 1993; Kocher et al., 1993; Schliewen et al., 1994; Kornfield & Parker, 1997; Verheyen et al., 1996, and references therein). The results have been stimulating to the field generally, have added significantly to our understanding of evolutionary modes and speciation mechanisms of the lake cichlids, and have served to highlight the important roles for both morphological and molecular analyses.

Another recent discovery of considerable interest and evolutionary implication is found in Ethiopia's Lake Tana, the highland source of the Blue Nile. In an elegant series of taxonomic and ecomorphological studies of trophic diversification, Nagelkerke and his colleagues have established the existence of an ecologically and reproductively segregated radiation of 14 species of the cyprinid genus *Barbus* (Fig. 20; Nagelkerke et al., 1994; Nagelkerke, 1997). Since the demise of the Lake Lanao cyprinid radiation in the Philippines, the Lake Tana flock becomes the planet's only remaining lacustrine cyprinid radiation.

Lest the impression remains that new discovery is limited to Africa's lakes, a few riverine examples serve to redress the balance. Recent work that combines neurobiology, behavior, and morphology has revealed remarkably rich communities of sympatric mormyrid taxa living in small rainforest rivers of west and central Africa (Hopkins, 1981; Moller, 1995). This is especially well illustrated for species in the genus *Brienomyrus* from Gabon (Fig. 21), where 11 new species have been discovered by recording distinctive electric organ discharges (Alves-Gomes & Hopkins, 1997) and detailed morphological study (Teugels & Hopkins, 1998; Teugels & Hopkins, in prep.). Mormyrids generate electric pulses many times per second for purposes of active electrolocation, and as a consequence species-typical and sex-specific signals are broadcast nearly continuously. The diversity of wave patterns among sympatric species suggests that individuals exploit differences in waveform, polarity, and duration to identify the species and sex of signalers. Playback experiments using computer-synthesized EODs have confirmed their importance in reproductive isolation and sex recognition (Hopkins & Bass, 1981). There are even individual differences in EODs that are sufficient to permit the tracking of individuals and their movements in small streams monitored daily (Friedman & Hopkins, 1996), thus enabling non-intrusive ecological monitoring of populations.

Electric catfishes (family Malapteruridae) are endemic to Africa and are one of the continent's ichthyological icons. They owe their common name to their peculiar ability to produce stunning discharg-

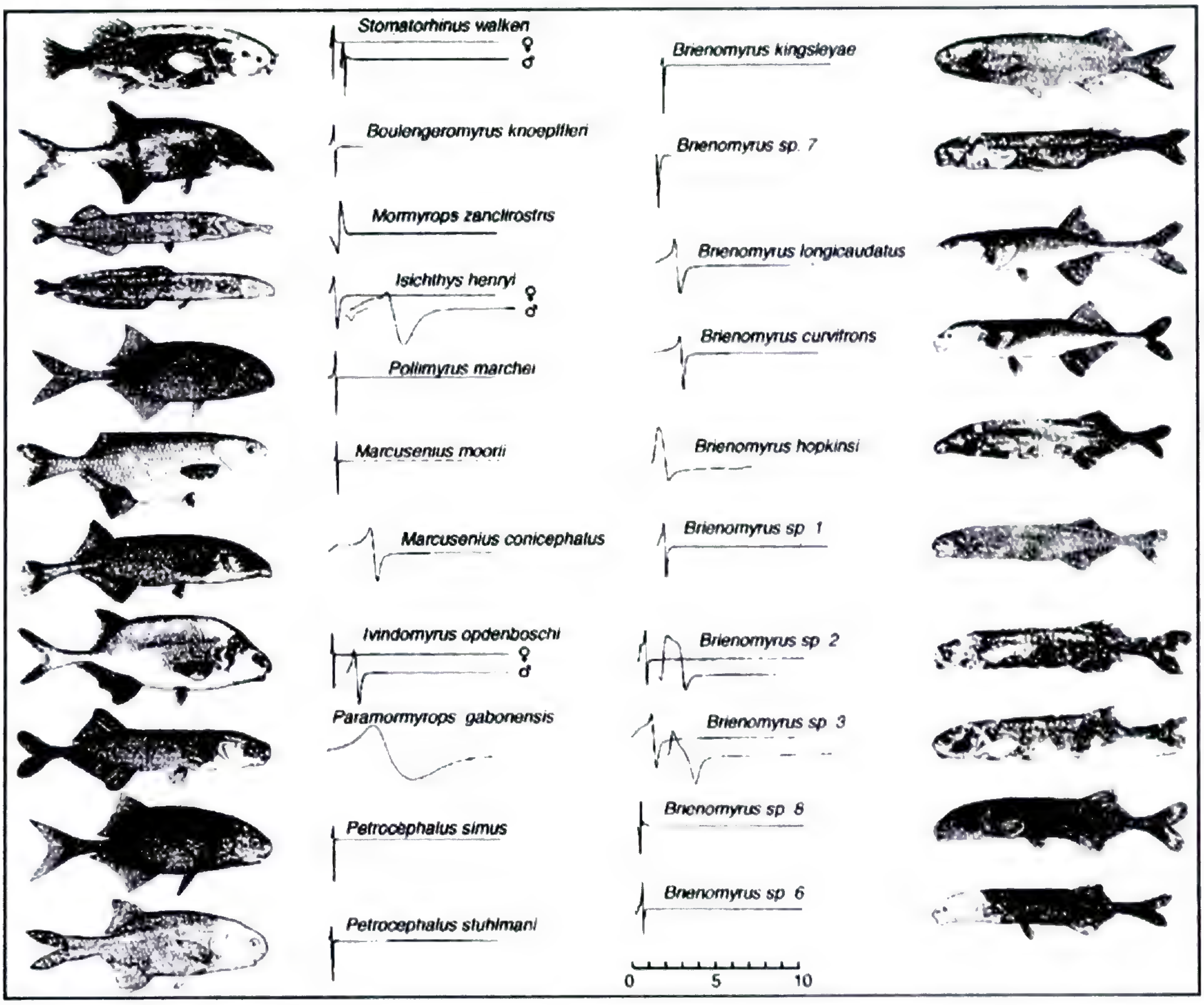


Figure 21. Mormyrid diversity in the Ivindo River (Gabon). Species-specific Electric Organ Discharges (EODs) arrayed in middle columns. (With permission and modified after Hopkins, 1981.)

es, up to 350V in large specimens. The first species was formally described 200 years ago as *Silurus* (now *Malapterurus*) *electricus*, and for much of the time since then a single species was recognized. However, electric catfishes are widely distributed across Africa, and careful revisionary work (Norris, in prep.) has recovered a complex of upward of 20 species previously unrecognized in museum collections and cataloged simply under the name *M. electricus*. Interestingly, the diversity of electric catfish species matches that of other groups conforming to the basic division of ichthyofaunal provinces of the continent. Beyond taxonomic diversity, Norris has uncovered a large amount of ecological and anatomical variety. For example, some taxa are highly depressed bottom-dwellers, others are fusiform and presumably active swimmers. By far the most astonishing discovery in this family is a clade of dwarf forms in which females can be gravid at sizes as small as 6 cm, a size range in which specimens of other species show little gonadal differentiation. Most of the dwarf catfishes have reduced cephalic and lateral line systems, elongate adipose fins, and a highly unusual three-chambered swim bladder. The function of the three-chambered bladder is as yet unclear, but Norris has suggested that it may play a role in the acoustic biology of these fishes, either in sound reception or production, or both.

There is little doubt that a similar taxonomic complexity will be revealed when other supposedly widespread "species," such as the enigmatic characoid *Hepsetus odoe*, are subject to the same sort of critical review. New species, and even whole new communities, abound in Africa, and their discovery and documentation will continue to require effort and render important results. But without doubt the greater scientific challenge will be the unraveling of the phylogenetic relationships of the African ichthyofauna. Such information is critical to our understanding of the evolutionary history of the fauna, the geologic history and relationships of the continent, and increasingly, as an aid in informing conservation work. In all these aspects, this is a task that has hardly begun.

The Malagasy region has long been recognized as a biotic entity distinct from that of mainland Africa. The region includes the Grande Ile of Madagascar and its offshore islands, the volcanic Mascarenes and Comores, and the granitic Seychelles. As noted by Roberts (1975) the region, which is something of an enigma to biogeographers, harbors a highly endemic ichthyofauna. More recently Stiassny and Raminosoa (1994) provided a review of the fauna and, in addition to recognizing many of the Malagasy endemics as occupying phylogenetically basal positions in their respective clades, they noted the enigmatic absence of a great many of the families currently represented in Africa and India. Because of its singular nature, the Malagasy ichthyofauna is not treated in detail in this essay. For more information on the Malagasy ichthyofauna see Teugels et al. (1985), Stiassny and Raminosoa (1994), and Benstead et al. (2000).

TROPICAL ASIA

As treated here Tropical Asia approximately equals the Oriental Realm, extending from the Indus basin eastward to South China and to the Moluccas (ca. "Wallace's Line") in Indonesia (Fig. 2). The dominant groups among primary and secondary freshwater families are Cyprinidae (about 1000 species), Balitoridae (about 300), Cobitidae (about 100), Bagridae (about 100), Osphronemidae (85); and among peripheral families, Gobiidae (about 300). This pattern applies principally to the mainland areas and in the insular parts to the major river basins draining onto the continental shelf. On oceanic islands and on the mainland in streams not draining to the continental shelf, the proportion of primary freshwater fishes is lower, and there is an increase in representation of peripheral families. In the Moluccas, Sulawesi, and most Philippine islands, there are no primary freshwater fishes, and gobies constitute about half of the inland fish fauna.

While recognizing an element of arbitrary taxonomic convention in doing so, fish families are often used as a rough guide to higher-level taxonomic diversity of faunas. A distinguishing feature of the Tropical Asian inland fish fauna is its high number of families. One-hundred-twenty-one families have been recorded from inland waters, either as permanent, temporary, or occasional residents (cf. 45–50 in Africa and about 55 in South America). Thirty-four of these families are primary or secondary division freshwater fishes, and 18 are endemic to Southeast or tropical Asia. The remaining 87 families are peripheral freshwater fishes, most represented by few species. The high number of peripheral families in Asian freshwater is explained by the existence in adjacent seas of the highest fish family diversity in the world. The northern tropical Asian ichthyofauna has some affinities with that of northern temperate Asia, with which it shares some cyprinid and cobitoid lineages, but on the whole, tropical Asia shares several lineages with Africa (e.g., Notopteridae, several cyprinid lineages, Bagridae, Schilbeidae, Aplocheilidae, Nandidae, Anabantidae, Channidae, Mastacembelidae).

An estimated total number of species for tropical Asian freshwater fishes is 3000. More precise figures have been compiled for some parts of Southeast Asia (Irrawaddy, Salween, Mekong, Red River basins and intermediate areas, Hainan, Malay Peninsula, the Philippines and Indonesia eastward to the Moluccas), where 2100 valid species are presently known (Kottelat, in prep.). However, considerable differences exist in our ichthyological knowledge among regions and countries because of incomplete surveying and variable ichthyological practices among countries.

The Indian ichthyofauna remains in need of in-depth systematic study. Recent work on Sri Lankan and southern Indian fishes indicates that many species thought to be widely distributed and conspecific with species originally described from northeastern India are in fact assemblages of allopatric species (Pethiyagoda, 1991; Pethiyagoda, pers. comm.; MK, pers. obs.). In Kerala (South India) 10–20% of the fishes in any basin of reasonable size are likely to be undescribed (Pethiyagoda & Kottelat, 1994). Habitat deterioration in this region poses a serious threat to most species, including those that are poorly known or unknown.

The development of ichthyology in China has been largely independent of that in the rest of Asia, but many scientific names applied to China's freshwater fishes are taken from adjacent faunas without adequate comparison. Survey work is very incomplete, especially in the hilly areas in the south. Species diversity is underestimated especially for small-sized fishes. For example, the loach identified as *Schistura fasciolata* is probably an assemblage of at least half a dozen species. Unfortunately, economic changes in recent years have made illegal electric fishing widely available everywhere. This poses a serious threat to the survival of most endemic hill stream fish communities. In 1996 one of us (MK) observed in western Sichuan that most small streams surveyed were without fish.

Knowledge of the ichthyofauna of tropical Asia is still in an exploration and discovery phase. The fish fauna of Laos jumped from 216 species recorded in the scientific literature as of early 1996

to some 370 known by mid-1997 (Kottelat, 1998). This represents an increase of about 80% in about 11 weeks of fieldwork. Some 85 of these species were unnamed, though many were known at the time of the fieldwork; about half of them are loaches of the family Balitoridae that inhabit hill streams and have a relatively small distribution area, often restricted to part of a single river basin. Similar figures can be recorded for most Asian countries (Kottelat & Whitten, 1996b). The situation is likely similar (but much more acute) in Vietnam, where, as in China, ichthyology has been largely isolated.

Survey efforts in little-known areas and poorly sampled habitats continue to yield new discoveries. Kottelat et al. (1993) listed 964 species known in 1992 from western Indonesian inland waters. An addendum published in 1996 added 79 species described in the interval, and others have been discovered but not yet reported. Changes in systematic or nomenclatural status affected 56 other species (Kottelat & Whitten, 1996a). Fieldwork in reasonably remote areas easily results in the discovery of 1 or 2 new species per actual day of fieldwork, but also in the discovery of an almost equal number of identification problems involving earlier known species.

"Sundaland" (Java, Borneo, Sumatra, Malay Peninsula) has a rich fauna (total ca. 1200 species). This is due to its historical fragmentation into smaller isolated basins and very diverse habitats ranging from coastal mangroves to high altitudes. One unusual habitat type has been discovered to harbor a very specialized and diverse fauna. This is the peat swamp forest, characterized by a peat-like soil (not made of sphagnum, but of tree roots) with a morphology somewhat similar to northern peat bogs, culminating into peat domes with a central "lake." The black water of these oligotrophic lakes is darkly tinted by tannin and has a very low pH (3 to 5). Once thought to be depauperate, there are now more than 100 species known exclusively from these habitats. Most are small fishes: fighting fishes (*Betta*), licorice gouramies (*Parosphromenus*), and numerous diminutive cyprinids. A particularly interesting discovery in the peat swamp is *Bihunichthys monopteroides* (Fig. 22), a chaudhuriid earthworm eel (Kottelat & Lim, 1994). This family was considered endemic to Inle Lake in Myanmar, but it is now known from about a dozen species with an overall wide range in Asia. *Bihunichthys monopteroides* is the smallest chaudhuriid (maximum known size of 36 mm SL) with extreme reduction or loss of scales, lateral line, fins, and a number of cranial bones. It has fossorial habits in soils around the swamps.

Figure 22. A diminutive, fossorial earthworm eel, *Bihunichthys monopteroides*, from an Asian blackwater swamp (photo by Peter K. L. Ng; drawing by Kelvin Lim).

In tropical Asia, as in Africa and South America, discovery of miniature species is fairly common. Many are cyprinids (Fig. 23B). *Danionella pellucida* from Myanmar and *Boraras micros* from northeast Thailand are adult at about 12 mm SL, and females of the loach *Kottelatlimia katik* are ripe at about 13 mm SL. In mangroves the smallest known species is an undescribed goby, *Gobiopterus* from Singapore, that reaches about 10 mm SL. Miniatures provide one of the most striking examples of discovery of a new fish clade, the family Sundasalangidae (Fig. 23C), described based on two species in 1981 by Roberts. These are tiny (25 mm SL) and transparent fishes with many reduced structures, and, in places, they are extremely abundant. Four additional species have been found on Borneo since their first discovery, and Siebert (1997) provided evidence that the group is related to herrings (Clupeidae).

At the other end of the size scale, occasional large-sized species are still being described. Some of these were reported by early explorers, but it is only in recent years that they have been carefully diagnosed and described. In Asia an impressive example is *Himantura chaophraya* (Fig. 23A), the Mekong sting ray known to science since 1880 but just named in 1990 (Monkolprasit & Roberts, 1990). This immense fish can attain a disc width of 2 m, an estimated total length of 4 m, and a weight of 600 kg.

Probably the most interesting communities of Southeast Asian fishes discovered in recent years are the species flocks of the lakes of central Sulawesi, Indonesia. A group of five tectonic lakes (Malili lakes) and the connecting streams host a fauna

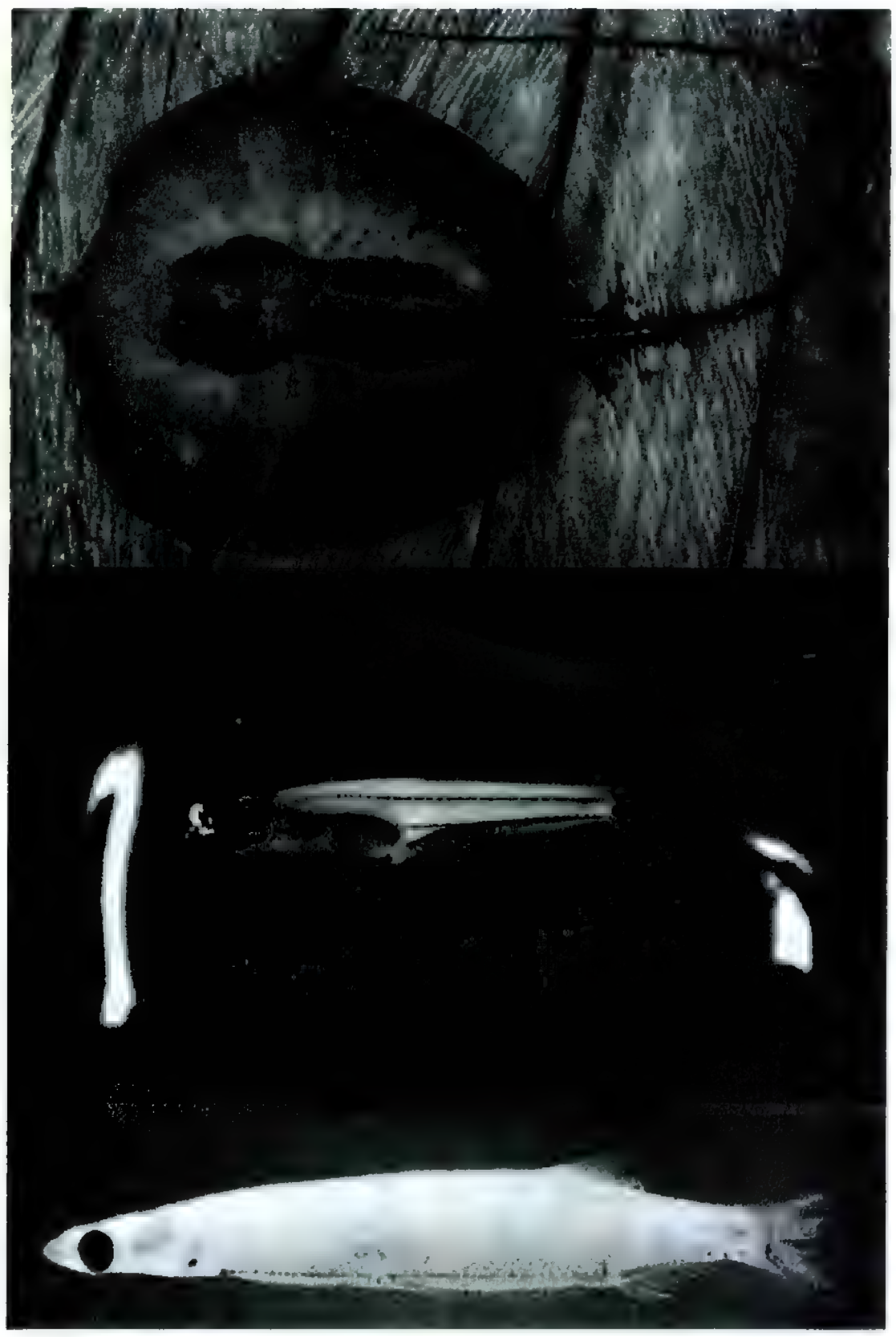


Figure 23. Recent discoveries of very large and small fishes in tropical Asia. —A. Freshwater sting ray, *Himantura chaophraya*. —B. A tiny, undescribed cyprinid, 9.9 mm long, from Borneo, Sumatra, Malay Peninsula. —C. One of the enigmatic noodlefishes, *Sundasalanx platyrhynchus*, 23.8 mm long. (Copyrights Maurice Kottelat.)

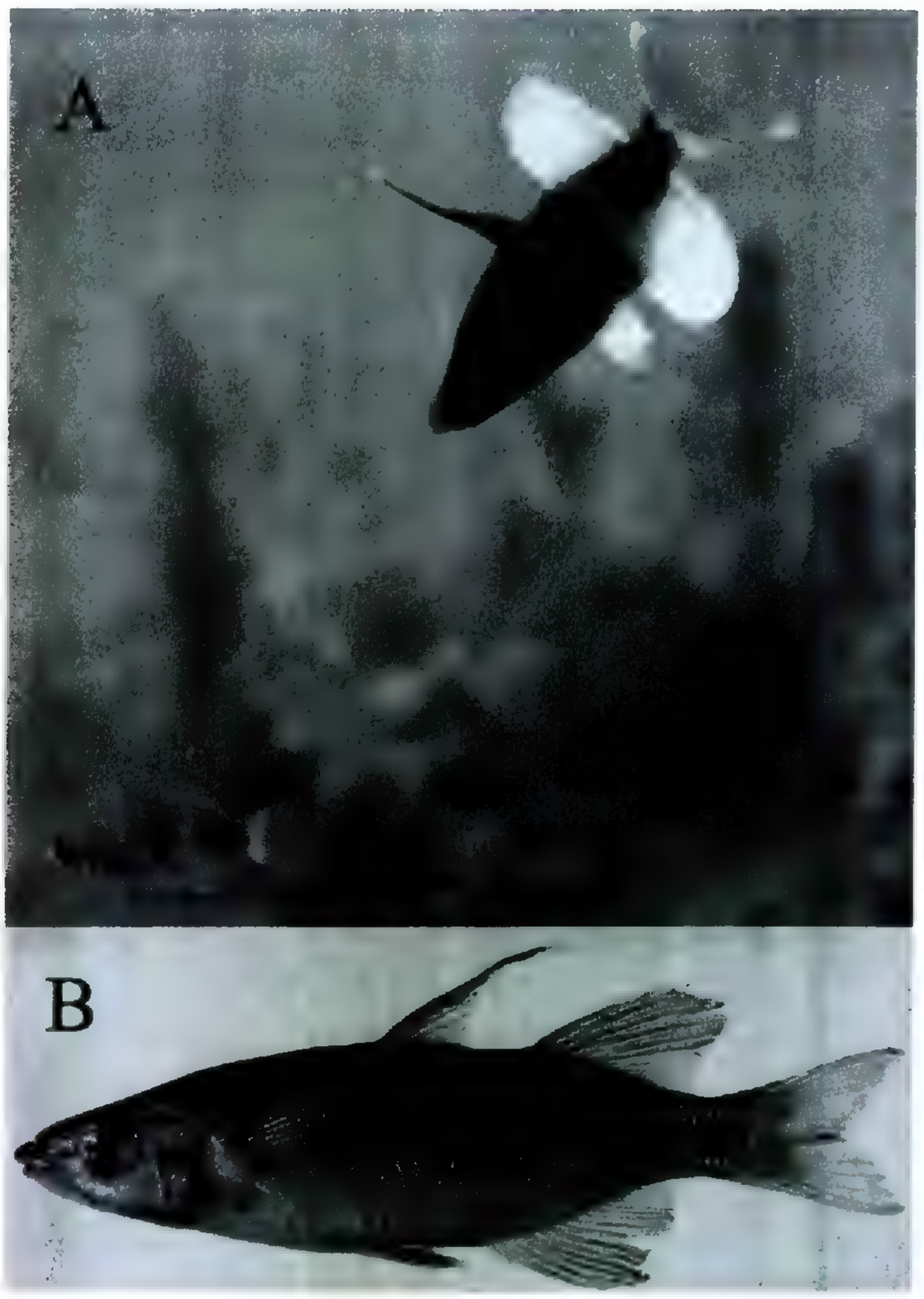


Figure 24. Endemic lake fishes of Sulawesi. —A. A courting pair of *Telmatherina antoniae*. —B. Fin-ray and scale-eating *Telmatherina prognatha*. (Copyrights Maurice Kottelat.)

consisting of 26 known native species, all but 4 endemic to this system. Within this lake system, two units can be distinguished, Lake Matano and lakes Towuti, Mahalona, and Wawontoa; only 2 endemic fish species are shared by these two units. The fish species include 3 endemic Hemiramphidae (halfbeaks), 6 endemic Gobiidae (gobies), 3 endemic Oryziidae (ricefishes), 14 endemic Telmatherinidae (sailfin silversides); also present are two endemic genera and 4 non-endemic species (one each in the families Telmatherinidae, Gobiidae, Synbranchidae, Anabantidae). The lakes also contain at least 4 endemic crabs, a dozen endemic shrimps, about 60 endemic molluscs, and endemic ostracods, sponges, other invertebrates, and macrophytes (Kottelat, 1990b, c, 1991; Larson & Kottelat, 1992; Bouchet, 1995).

The family Telmatherinidae includes five genera, two endemic to the lakes, one with eight species endemic to the lakes plus one riverine species occurring also in an adjacent basin, one monotypic genus endemic to southwestern Sulawesi, and another monotypic genus from Misool, an island off Irian Jaya (the Indonesian part of New Guinea) (Aarn et al., 1998). While the larvae of all telmatherinids are "pelagic," the adults live close to the bottom and are substrate spawners. They are strongly sexually dimorphic (Fig. 24A), and males of several species exhibit color polymorphism. One species (*Telmatherina prognatha*, Fig. 24B) apparently feeds on fin-rays and scales of other species, and another (*T. sarasinorum*) preys on eggs of other species.

The other lakes of central Sulawesi, lakes Poso and Lindu, have less diverse fish faunas. Lake Lindu apparently hosts a single native species, *Xenopoecilus sarasinorum* (Adrianichthyidae), while Lake Poso is inhabited by two endemic Gobiidae, three endemic Adrianichthyidae, and two endemic Oryziidae (Kottelat, 1990a). The Adrianichthyidae are noteworthy for their breeding behavior. Although originally recorded as live-bearers, it now appears (Fig. 25A) that the female carries a clutch of eggs with her pelvic fins, in a ventral depression of the belly, for about 10 days until they hatch.

Adrianichthyidae are closely related to Oryziidae, which are known from India to Japan and Ti-

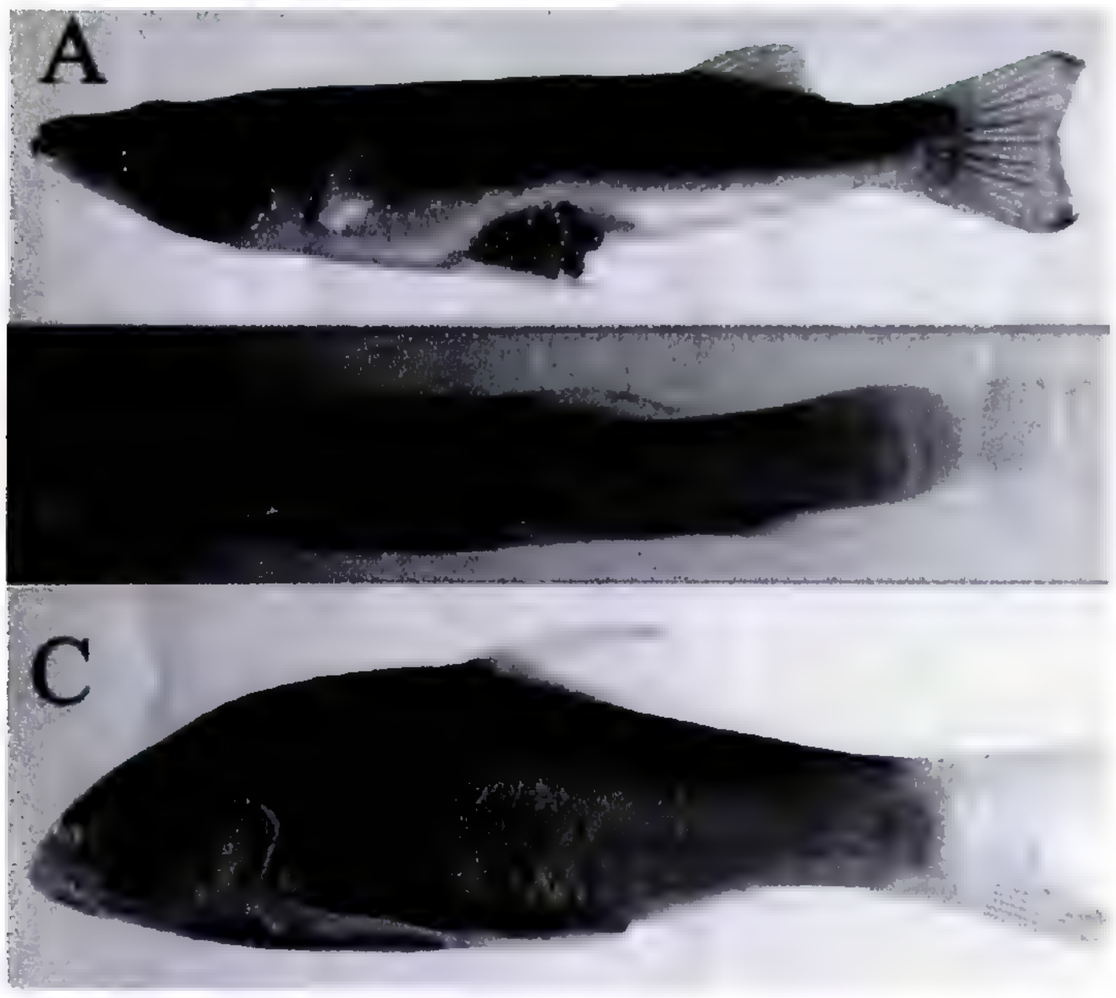


Figure 25. Recent discoveries of oddly specialized fishes in tropical Asia. —A. The adrianichthyid *Xenopoecilius oophorus* with a fertilized clutch of eggs. —B. The cave-dwelling balitorid loach *Schistura oedipus* from Thailand. —C. The cave-dwelling cyprinid *Puntius* sp. from Java. (Copyrights Maurice Kottelat.)

mor, in fresh and brackish waters. Interestingly, Adrianichthyidae are known on Sulawesi from that part of the island (the western half) that plate tectonics suggest was earlier connected to elements now constituting part of Southeast Asia. On the other hand, the distribution of telmatherinid species (except one) on the eastern half of Sulawesi corresponds to a plate earlier connected with the New Guinean one, on which Misool is located, where the family is also represented by an endemic genus (Kottelat, 1991).

Tropical Asia contains several of the most extensive karst areas of the world, and it is not surprising that at least 31 species (some undescribed) of cave fishes are known from this region and many more should be expected. Some of the largest karsts (Laos, Vietnam) have barely been explored speleologically. Sixteen species of cave fishes are known from China, seven from Thailand, four from India (three actually from wells), two from Indonesia, and one each from Malaysia and Laos. Most of these belong to genera known from surface waters, but five cannot be linked with known surface species and are treated as distinct genera. Noteworthy is the cyprinid genus *Sinocyclocheilus*, which has evolved independently at least seven hypogean species in China (Guizhou, Yunnan, and Guangxi provinces). The epigean species of *Sinocyclocheilus* are usually found associated with springs or in streams under overhanging rocky shores, which make them "pre-adapted" to colonize cave habitats. The known cave fishes in Asia belong to the families Balitoridae (13 species, e.g., Fig. 25B), Cyprinidae (12, e.g., Fig. 25C), Cobitidae (2), Synbranchidae (2), Gobiidae (1), and Clariidae (1).

AUSTRALIA AND NEW GUINEA

Australia and New Guinea are perhaps better known for their diverse marine fish faunas than for their freshwater ones. Nevertheless, freshwater fishes of Australia and New Guinea are distinctive, interesting, and have been the subject of significant ichthyological discovery over the past 20 or so years.

With an area roughly equal to the contiguous continental U.S.A., Australia (Fig. 2) is generally characterized by lower topographical relief, lower rainfall, and a much less extensive system of river drainages. However, largely as a consequence of the latitudinal spread of the country (from about 10°40′S to about 43°40′S), and of the influence of the Great Dividing Range on climatic conditions, Australia possesses a wide variety of freshwater habitats. These include artesian springs and ephemeral desert lakes and streams (Great Australian Bight, Lake Eyre, and other internal drainages); tropical streams that undergo extensive flooding during summer monsoonal rains (northwestern Australia and Gulf of Carpentaria drainages); rainforest streams (northeast coast); coastal streams and sand-dune lakes (east coast); and alpine lakes (Tasmania). The Murray-Darling River System is by far the largest in Australia, extending southwestward about 1900 km from the interior of southern Queensland to the Southern Ocean.

Stretching around 1600 km, New Guinea is the world's second-largest island (Fig. 2). Biogeographically and geologically, New Guinea is divided into more-or-less northern and southern provinces, separated by an extensive mountain chain along the long axis of the island. This mountainous topography, in combination with high rainfall, results in numerous drainage systems, and a large array of freshwater habitats that include: short coastal streams, large lowland rivers, coastal swamps and floodplain lakes, alpine streams and lakes, and large highland rivers.

Australia and New Guinea are much more than just adjacent land masses, as they have been connected throughout most of their history. The Sahul Shelf, beneath the shallow Arafura Sea and Torres Strait that now separate the two areas, was emergent until as recently as about 6000 years ago during the latest glacial lowering of sea level, and southern New Guinean streams were confluent with those of the adjacent Australian coast.

Australian freshwater fishes were reviewed by Allen (1989), who recorded 187 species and subspecies; subsequent collecting and research have taken this count to around 215. The dominant freshwater taxa in the country are the gobioid families Eleotrididae and Gobiidae (about 50 species), the superfamily Galaxioidea, an austral group of salmoniform fishes related to the northern smelts (26 species), Terapontidae, an Indo-West Pacific group of perch-like fishes (22 species), Percichthyidae, a family of southern Australian and southern South American perch-like fishes (21 species), Melanotaeniidae, an Australian–New Guinean endemic family (but, see below) of atherinomorph fishes (16 species and subspecies), Plotosidae, an Indo-West Pacific catfish family (about 15 species), and Atherinidae, a worldwide atherinomorph family (15 species and subspecies). Other distinctive components include the Queensland lungfish, *Neoceratodus forsteri* (Neoceratodontidae), and the bony-tongue (osteoglossid) species, *Scleropages jardinii* and *S. leichardti*.

Freshwater fishes of New Guinea were reviewed by Allen (1991), who listed 320 species, including some estuarine forms; subsequent collecting and research have taken this count to about 350. The most diverse freshwater taxa are eleotridids and gobiids (about 115 species), Melanotaeniidae (53 species), Ariidae, a circumtropical catfish family (21 species), Terapontidae (16 species), Chandidae (= Ambassidae), an Indo-West Pacific family of small, perch-like fishes (15 species), Pseudomugilidae, a family of atherinomorph fishes endemic to Australia and New Guinea (13 species), and Plotosidae (about 13 species). Most of these taxa are also species-rich in northern Australia, emphasizing the historical link between the areas; indeed, about 50 species from southern New Guinea also occur in northern Australia, and many of these are restricted to the two areas.

The amount of recent ichthyological activity in Australia and New Guinea can be gauged conservatively from the number of recently described species and subspecies. Since 1970 about 70 Australian (33% of the total) and about 125 New Guinean (36% of the total) freshwater fish species and subspecies were described or are awaiting description.

Increased understanding of freshwater fish systematics and distributions in the region has largely stemmed from the application of modern collecting and systematic techniques by professional ichthyologists. However, no less significant has been the input from amateur ichthyologists and aquarists. In particular, during the last three decades a profound increase in interest in keeping freshwater fishes has

Figure 26. Some of the taxonomically challenging and diverse rainbowfishes of Australia and New Guinea. —A. *Melanotaenia maccullochi*, typical ("Cairns") form lacking distal stripe on anal fin, 53.0 mm long. —B. *Melanotaenia maccullochi*, "New Guinea" form with distal stripe on anal fin, 33.0 mm long.

resulted in the formation of various specialty societies (e.g., Australia New Guinea Fish Association, "ANGFA") and in numerous amateur collecting expeditions. Aquarist interest has particularly concentrated on the endemic rainbowfishes (Melanotaeniidae, Fig. 26), and this, in combination with taxonomic and field studies by G. R. Allen, has resulted in a dramatic increase in the number of species recognized in this family: of the 67 species and subspecies currently recognized, 38 (57%) are either undescribed or were described since 1970.

Other significant new discoveries in recent years have included: *Oxyeleotris caeca* (Fig. 27A), a blind butidid gobioid from sink holes and caves in the Upper Kikori River, Papua New Guinea (Allen, 1996); two new giant percichthyids of the genus *Maccullochella* from coastal drainages of southern Queensland and northern New South Wales (Rowland, 1993); *Scaturiginichthys vermeilipinnis*, a new genus and species of pseudomugilid from an artesian spring in central western Queensland (Invantsoff et al., 1991); four new gobiids of the genus *Chlamydogobius* (Fig. 27B) from artesian spring systems in central Australia (Larson, 1995); and numerous lacustrine atherinid, eleotridid, and melatotaeniid species endemic to various lake systems in New Guinea (e.g., Allen & Hoese, 1986; Crowley et al., 1995; Allen, 1995, 1998; Allen & Renyaan, 1996).

There also have been significant advances in our understanding of the phylogenetic relationships of Australian and New Guinean freshwater fishes. For example, Johnson (1984) diagnosed a monophyletic, freshwater Percichthyidae that included the southern South American genera *Percichthys* and

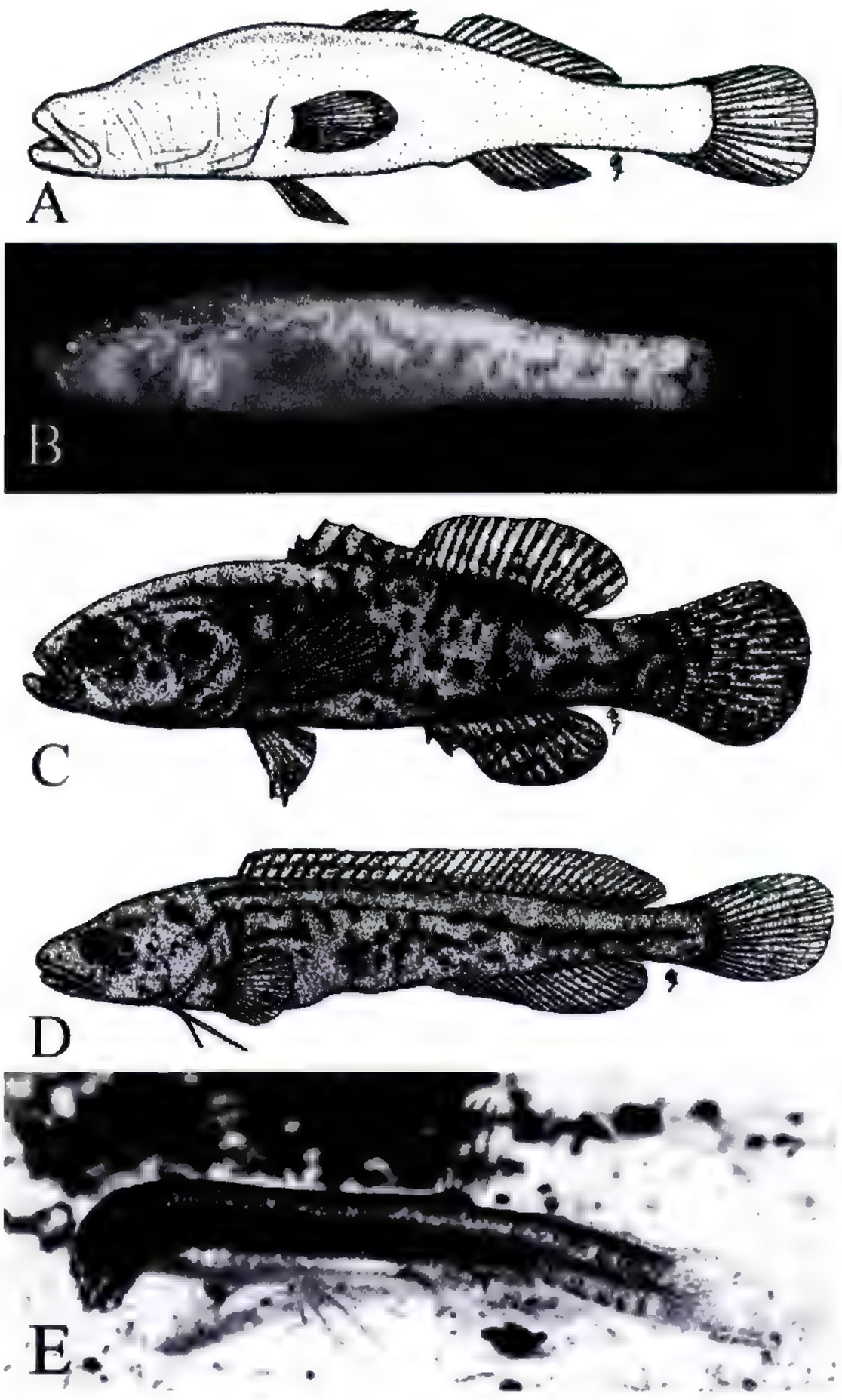


Figure 27. A potpourri of recent ichthyological discoveries in the Australia–New Guinea region. —A. The cave-dwelling gobioid, *Oxyeleotris caeca*, 107 mm long, from Papua New Guinea (based on Allen, 1996). —B. One of the artesian spring-dwelling gobioids, *Chlamydogobius japala*, 35.5 mm long, Northern Territory, Australia. —C. *Bostockia porosa* (based on Merrick & Schmida, 1984). —D. *Gadopsis marmoratus* (based on Merrick & Schmida, 1984). —E. The burrowing salamanderfish, *Lepidogalaxias salamandroides*, from Western Australia (copyright by Tim Berra).

Percilia, and the southern Australian genera *Bostockia* (Fig. 27C), *Gadopsis* (Fig. 27D), *Edelia*, *Nannoperca*, *Nannatherina*, *Maccullochella*, *Percalates*, *Plectroplites*, and *Macquaria* (the latter three combined into a probably paraphyletic *Macquaria* by MacDonald (1978) and most recent authors). Previously (e.g., Gosline, 1966), the Percichthyidae had been a catchall for basal perciform taxa that included, among others, marine genera now assigned to the Acropomatidae, Serranidae, Moronidae, and Polyprionidae. Johnson further hypothesized that the southeastern Australian *Gadopsis* is sister to the southwestern Australian *Bostockia*, and that these genera form a clade with the diminutive *Edelia*, *Nannatherina*, and *Nannoperca* (previously classified in the Kuhliidae). Prior to this, the relationships of *Gadopsis* were enigmatic, and it had been variously placed in its own order (Scott, 1962), allied to ophidioids (Gosline, 1968), allocated to a monotypic percoid superfamily (Nelson, 1976), or suggested as a possible relative of the perciform suborders Trachinoidei or Blennioidei (Rosen & Patterson, 1969).

No Australian freshwater fish has attracted as much attention from systematists as the southwestern Australian salamanderfish, *Lepidogalaxias salamandroides* (Fig. 27E). Originally described as a galaxiid (Mees, 1961), subsequent authors have either retained it within the Galaxiidae (or at least within the austral taxon Galaxioidei), or suggested radically different positions for the taxon, such as the sister of a clade consisting of the Northern Hemisphere freshwater families Esocidae and Umbridae (Rosen, 1974), or in an unresolved trichotomy with the Salmonidae and Neoteleostei (Fink, 1984). However, recent studies have nested the genus within the galaxioids. Johnson and Patterson (1996) concluded that *Lepidogalaxias* is the sister of the Tasmanian genus *Lovettia*, these together forming the sister of a clade consisting of the typical Galaxiidae plus the southern South American genus *Aplochiton*. Williams (1997) concluded that *Lepidogalaxias* is the sister of a clade consisting of the typical Galaxiidae and the Aplochitonidae (the latter including *Aplochiton* + *Lovettia*).

No less controversial in recent years has been the position and composition of the family Melanotaeniidae. This has particularly centered around its relationships to other atheriniforms, notably to the Australian–New Guinean family Pseudomugilidae, the western Indonesian and New Guinean family Telmatherinidae, the Madagascan family Bedotiidae, the southeast Asian Phallostethidae, and the West Pacific Dentatherinidae (e.g., Allen, 1980; Parenti, 1984b; Stiassny, 1990; Saeed et al., 1994; Dyer & Chernoff, 1996; Aarn & Ivantsoff, 1997).

There has been a substantial increase in our understanding of the biology of Australian and New Guinean freshwater fishes in recent years. Particularly noteworthy have been several studies on the biology of the aestivating galaxioid *Lepidogalaxias salamandroides* (reviewed by Berra & Pusey, 1997).

Unfortunately, two unwarranted assumptions about the nature of Australian–New Guinean freshwater fishes prevail in recent literature (e.g., Allen, 1989; Pollard et al., 1990; Berra, 1998): (i) the fauna is highly impoverished; and (ii) it is dominated by species that have recently evolved from marine ancestors. Both serve to devalue the significance of the fauna, and both are unquestionably premature.

For example, Allen (1989: 8) noted that Australia has remarkably fewer freshwater species than does the continental U.S.A. However, no less remarkable is the difference in effort devoted to the taxonomy of the two faunas.

Clearly, there is need for much more survey work to be done in Australia and New Guinea, as some areas remain poorly collected (particularly in Irian Jaya). However, perhaps more important, there is a need for more careful study of the many widespread species, as it is highly likely that such study will lead to a significant increase in the number of recognized species. For example, geographic variation has been reported in various Australian freshwater fishes, including representatives of the Atherinidae, Eleotrididae, Galaxiidae, Latidae, Plotosidae, Melanotaenidae, Terapontidae, Percichthyidae, Retropinnidae, and Pseudomugilidae. Indeed, one melanotaeniid species, *Melanotaenia trifasciata,* has been divided into as many as 35 geographic forms, each with highly restricted, allopatric distributions (Hieronimus, 1998).

While it is clear that the taxonomic reevaluation of widespread species will benefit from recently developed molecular techniques (e.g., Rowland, 1993; Musyl & Keenan, 1996; Jerry & Woodland, 1997), it is likely that the most significant advances will not come directly from technology, but rather from a sounder philosophical view of species and associated systematic method. Much could be gained from careful analysis of many morphological characters already at hand, such as coloration characters noted for many of the melanotaeniid "varieties." (Coloration characters, when not supported by other characters, have generally been dismissed by ichthyologists working on Australian and New Guinean freshwater fishes, although such premature judgment is unjustified; a similar problem exists for Indo-Pacific shore fishes; see Gill, 1999.) There is urgent need for such studies in order that species be properly conserved and managed.

One could argue about the biogeographic relevance of whether the freshwater fishes of Australia and New Guinea are of marine ancestry or not, as it is incorrect to equate a marine habit with mobility and a random biogeography. Indeed, there are many endemic Australian marine fish taxa. However, even if marine ancestry was an issue, the assumption that Australian and New Guinean freshwater fishes are recent invaders from the sea is simply not supported by available evidence. While it is true that many of the dominant freshwater taxa also include marine species (e.g., Plotosidae, Terapontidae, and Gobioidei), it does not automatically follow that a marine habit is ancestral. Consider, for example, Vari's (1978: fig. 9) phylogeny of the Terapontidae. Most of the genera are exclusively freshwater and restricted to Australia, New Guinea, and several other Gondwanaland fragments in the Indo-Australian area. Three of the four genera with marine species (*Mesopristes, Terapon,* and *Pelates*) are relatively widely distributed in the Indo-Pacific, but occupy relatively derived positions in the phylogeny. The phylogenetic position of the fourth "marine" genus (*Amniataba,* which includes two species, an Australian freshwater species, and a southern New Guinean and Australian euryhaline species) is unresolved, but it could be near basal. *Leipotherapon,* the basal-most genus in the phylogeny, is exclusively freshwater. Therefore, one interpretation of available evidence, following a center of origin concept, suggests that terapontids are freshwater fishes, with several relatively recently evolved marine species.

Whereas many previous authors have acknowledged that a small component of the Australian–New Guinean freshwater fish fauna probably has a relationship with Gondwanaland (e.g., galaxioids, and the osteoglossid genus *Scleropages*), there is no justification for *a priori* rejection of such a relationship for the other taxa. For example, although the Australian Percichthyidae are often cited as being recently evolved marine invaders, this is largely a consequence of the previously loose definition of the family, and Johnson's (1984) cladistic definition of the family (see above) suggests that percichthyids, too, predate fragmentation of Gondwanaland.

Unfortunately, phylogenetic information is lacking for most Australian and New Guinean freshwater fish taxa, but the need for such studies is obvious.

In summary, the past few decades have seen significant advances in our understanding of the systematics, biology, and distribution of Australian and New Guinean freshwater fishes. However, there is need for continued work, particularly in the reevaluation of purported widespread species and in phylogenetic studies. There is every reason to predict that such studies will lead to a significant increase in recognized diversity, and a conclusion that the freshwater fishes are just as distinctive and historically/biogeographically informative as marsupials, birds, and the other, more famous components of Australia and New Guinea's biota.

Discussion

These are exciting times of discovery in freshwater ichthyology. In tropical freshwaters, new fish

species are being found and described at rates as high as any in the history of ichthyological exploration. Many recently discovered fishes exhibit highly unusual characteristics, and some represent previously unknown lineages (described as new genera and family-group taxa). New fish species are still found occasionally in Europe and North America, where the ichthyofaunas are already well documented. Phylogenetic studies of fishes from all over the world commonly reveal unsuspected higher-level evolutionary and biogeographical relationships. Investigations of even long-known fish species continue to discover novel characteristics and strange life histories. In addition, as the extent and patterns of fish diversity and relationships are more thoroughly documented, we are better able to determine the correlates and causes of fish diversification in evolutionary time.

These are also times of serious concern for the present and future health of populations, species, and communities of freshwater fishes and other aquatic organisms (e.g., Stiassny, 1999). A recent analysis of the North American situation by Ricciardi and Rasmussen (1999) estimates the extinction rate for freshwater animals at five times the terrestrial fauna and similar to that for tropical rainforests. Many human activities are increasingly disturbing and, in cases, destroying freshwater biotas (McAllister et al., 1985; Minckley & Deacon, 1991; Warren & Burr, 1994; Harrison & Stiassny, 1999). Wherever human populations are large or expanding, so increase dams and impoundments, dredged and straightened channels, erosional runoff and turbation, and the harmful waste products of mining, industry, agriculture, and urban growth. These impacts have unintended but negative and sometimes devastating effects on aquatic habitats and life. Consider the vast extent of interruption of natural river flows and fish movement, and altered benthic habitat and community structure that has already attended the proliferation of dams. According to the World Commission on Dams (1998), in 1997 there were an estimated 800,000 in the world, and "more than 45,000 of these dams have been categorized as large (dam height more than 15 meters above the natural river bed)." Among other horrific aspects of an ecological catastrophe centered on freshwater, witness, in the years following diversion of the Amu Darya and Syr Darya rivers, the total collapse of the Aral Sea biota (including a fishery that once produced 45,000 metric tons per annum).

Freshwater fishes are important and valued resources for food, sport, ornament, and biological control. Like so many other resources, populations and some species of freshwater fishes are already overexploited. Well-documented cases of overfishing include several species in the Laurentian Great Lakes (S. Smith, 1968) and the large pimelodid river catfishes of the Amazon (Barthem & Goulding, 1998). Freshwater fish communities and species also have been placed in harm's way by introductions of non-native species. Here, too, the problem is current and global in extent. Some of the best known examples are the sea lamprey in the Laurentian Great Lakes, Nile perch in Lake Victoria, African tilapia cichlids and American largemouth bass in many parts of the world, and trouts in Andean rivers. The world's most spectacular species flock of cyprinid fishes in Lake Lanao in the Philippines went to extinction following introduction of several exotics. Lake Dianchi in Yunnan, China, formerly held about 25 native species (12 endemic in the lake). About 40 species live there now, but most are introduced. All the lacustrine endemics are gone, including the monotypic *Sphaerophysa dianchiensis*, which was described about 25 years after the last living specimen was documented.

Human dependencies on and benefits from aquatic species and the communities and biotas they form are many, and some are critical, including food and indicators of water quality. Daunting and increasing as the threats are to freshwater biotas around the world, efforts must be made to reduce and mitigate their impacts by reducing habitat destruction, over-fishing, and introduction of alien species.

To meet the immense challenges confronting aquatic conservation biology, an accurate and thorough baseline knowledge of species-level diversity is essential. Such knowledge is also fundamental for research in systematics, ecology, and other sciences. The freshwater fish faunas reviewed herein exemplify how much we are still discovering about species and higher-level diversity, and suggest that much more remains to be discovered. Fishes are, of course, among the better known components of freshwater biotas. Even so the pace of habitat change is so rapid that it is likely that some, possibly many, freshwater fish species will disappear before they are known to exist. What else will disappear along with those unknown casualties? How much do we not yet know of the species of aquatic invertebrates, green plants, fungi, protists, and prokaryotes? We do not know, and possibly will never know, quite a lot of these organisms and the roles they play(ed) in their communities.

Fishes, because they are better known and relatively easy to monitor, serve as indicator organisms to monitor the health of aquatic ecosystems. But to achieve the needed baseline knowledge of Earth's

freshwater fish species will require much work. There are praiseworthy and productive programs that support biotic surveys and discovery, e.g., the US NSF Biotic Survey and Inventories Program. Some natural history museums, governments, and universities promote the enterprise, but the task remaining will depend on the continuing dedicated efforts of many individual ichthyologists in exploration, discovery, and description. The task remaining to make the world's fishes known is a big one. Casting, as we must, the larger taxonomic net to capture knowledge of Earth's entire biota is an immense and urgently needed job, but one that will yield a uniquely wonderful result.

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NEW MAMMALS IN THE 21st CENTURY?

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Abstract

After 50 years in which only one new large mammal had been found worldwide, three new ungulates were found in the same region of Vietnam within 4 years. The context of the finds is discussed in relation to the continuing finds of other mammals and birds. This paper draws conclusions about the types of places that may still conceal undiscovered mammals and predicts where future finds may be made into the next century.

Key words: Bovidae, Cervidae, Laos, mammals, *Megamuntiacus*, *Muntiacus*, muntjac, *Pseudoryx*, Saola, Vietnam.

New Mammal Finds among Scars of War

Out of the several million species of animals known to inhabit this planet, only a small number are mammals (just over 4600) and birds (11,000); yet these are the creatures the general public best know, like, and show most concern for. That there remain over 10 million microscopic insects to discover and name may fascinate biologists, but this does not make the newspaper headlines. Taxonomists see the job of describing the varied life forms of our planet as incomplete. The general public see it as a job nearly finished with just a few last hidden creatures still to be found.

Ever since Linnaeus began describing and counting the species with which Man shares the Earth, the number of known species has continued to grow. For some groups, the species discovery curve continues to rise ever steeper, but for the warm-blooded vertebrates, especially the large mammals, the curve is leveling off (Medellín & Soberón, 1999), and we can hazard some guesses as to how many more species there are still to find. Medellín and Soberón (1998) estimated another 247 mammals, mostly small, will be discovered between 1992 and 2032.

During the first three decades of this century, only a handful of new large mammals were discovered. The finding of okapi, *Okapia johnstoni*, in 1901 in the forests of Congo created enormous popular interest and speculation that this was the last great new mammal. But other new African finds were quickly made: giant forest hog, *Hylochoerus meinertzhageni*, in 1904, and Mountain Nyala, *Tragelaphus buxtoni*, in 1910, before the African vein started to dry up. In 1937, Urbain described the Kouprey, *Novibos sauveli*, a large ox of Cambodia, actually found in a zoo in Paris. During the next 50 years, only one new large mammal was found. The Chaco Peccary, *Catagonus wagneri*, already known and named from fossils, was found to be still extant (Wetzel et al., 1975). A few new species were added by taxonomists splitting known forms: African colobine monkeys, Sulawesi macaques. Some zoologists believed the large mammals were, by and large, all in the museum and cataloged.

Discoveries of new birds have been equally scarce. Ninety-eight percent of all 1135 Palearctic birds were described between 1758 and 1900. Only seven new Palearctic birds have been described since 1920, with five of these being in the less-explored regions of China (Roselaar, 1994). Moreover, three of the seven finds lay for years unrecognized in museum collections before being recognized as new: pink-rumped rosefinch, *Carpodacus eos*, Vaurie's nightjar, *Caprimulgus centralasicus*, and Sillem's mountain finch, *Leucoslicte sillemi*. The last had been collected 62 years before being recognized as new in the Museum of Amsterdam (Roselaar, 1994).

In 1992, a completely new, large mammal was found in the North Annam mountains in the Vu Quang Nature Reserve of Vietnam: the Saola or Vu Quang ox. Assigned to its own genus, *Pseudoryx*, DNA showed the ox was a primitive member of the cow and goat family Bovidae (Dung et al., 1993). Two sets of the unique horns were initially found hanging as a hunter's trophies in a Vietnamese village. Subsequent morphological and genetic studies have shown the animal is so unlike anything else that it should be regarded as a new subfamily. A great amount of media interest was devoted to the find, but it took two years before anyone actually saw the animal alive, when two young animals were caught by farmers and brought to Hanoi.

The find was exciting for several reasons. It caused a major revision of the taxonomy of the fam-

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ily Bovidae. It helped highlight the conservation status of the neglected Annam Mountains Region. But most of all, it made people realize we really do not know all our large mammals, and it is still worth looking for more.

Subsequent surveys in Vu Quang turned up a second new ungulate, the giant muntjac, *Megamuntiacus vuquangensis* (Tuoc et al., 1994). The finds were made in the same village as was found the type specimen of the Saola. This muntjac species was initially given a new genus name because it did not match the existing genus description for *Muntiacus* (Groves & Grubb, 1990). Further DNA studies have shown the new genus may be insufficiently distinct to warrant its own genus (Giao et al., 1998). There is some argument (Schaller & Vrba, 1996; Groves & Dawson, in press) as to whether *Muntiacus* should be redefined to accommodate the giant muntjac. This cervid mammal differs from the other seven known muntjacs by its larger size, short pedicles, and much larger, more spreading antlers.

Both Saola and giant muntjac were also found in Laos, with wider searches in the Annamite Mountains revealing a new pygmy muntjac, *Muntiacus truongsonensis* (Giao et al., 1998). In Laos, the Roosevelt's muntjac (previously known from only one specimen) was rediscovered together with a bearded pig, *Sus bucculentus* (previously known from only one lost specimen in Shanghai Museum) (Schaller & Vbra, 1996). A new striped rabbit was also found in Laos (formerly thought to be a Sumatran endemic genus) and has now also been found in Vu Quang and Pumat Reserves in Vietnam, and still awaits scientific description. Another small muntjac has also been found in the central Annam Mountains of Vietnam, awaiting analysis and description (Hulse, pers. comm.).

Vu Quang, itself in a remote evergreen part of the North Annam Mountains on the border between Vietnam and Laos, continues to reveal novelties. Two new species of fish have just been described there (WWF, 1996).

North Annam was already recognized as a small pocket of local endemism with such local specialities as Owston's palm civet, *Chrotogale owstoni*, Hatinh leaf monkey, *Semnopithecus (francoisi) hatinhensis*, Vietnamese pheasant, *Lophura hatinhensis*, and sooty babbler, *Stachyris herberti*. These new finds of undescribed mammals add significantly to the biological and conservation importance of this region.

These exciting new discoveries in Vietnam and Laos seemed to stimulate a new wave of search and discovery around the world. A new antelope, *Pseudonovibos spiralis*, was described from Cambodia on the basis of several sets of unique spiraling horns (Peter & Feiler, 1994). But the failure of efforts to find the animal alive suggest we may be too late to see or save this species. A new tree kangaroo (the bondegezou) was discovered in the Jayawijaya Mountains of Indonesian New Guinea. No less than seven new marmosets (the latest being *Callithrix mauesi* and *C. nigriceps*) were added to the mammals list in Brazil. Two new bushbabies, *Galaoides rondoensis* and *G. udzungwensis* (Kingdon, 1997), have been described from Tanzania. A new horse was reported in the popular press from Xinjiang, China.

Are we on a new wave of discovery? In fact, the discovery of new mammals has been rather steady throughout the century. More than a hundred new mammals have been described without attracting much public attention (Wilson & Reeder, 1994). These have been largely bats, rodents, and insectivores, or cases of splitting up previously recognized taxa such as the Sulawesi macaques and Sulawesi tarsiers.

Where Will Future Finds Be Made?

The Annam Mountains of Vietnam and Laos do seem to be a rich, and still not fully explored, source of diversity. It is one of the world's overlooked "biodiversity hotspots." The spectacular mammalian finds are largely due to a time warp in an area where zoological exploration had been held up for 50 years due to constant warfare and political trouble. But these finds do give us clues about where to find yet more new species.

Despite being in one of the most populated regions of the earth and a region heavily devastated by both chemical and physical bombardment during the Vietnam War, the North Annam Mountains are rugged, difficult to access, unattractive for agriculture, and ecologically isolated from much drier surrounding lowland forests. Highland peaks are small and separate, resembling a small archipelago of evergreen montane islands. The region is part of an evergreen tropical continental system that has enjoyed climatic stability for thousands of years and where climatic oscillations of the Pleistocene could be easily accommodated by species by making minor vertical movement in the steep terrain (Giao et al., 1998). These are conditions ideal for the creation of local endemic species as well as for the survival of primitive and relict forms. The region is both a classic Pleistocene refuge and a source of new vertebrate radiation.

The following key characters can be identified as

indicators of the likelihood of a given geographic area still hiding undiscovered forms:

(1) area of long geological stability;
(2) area of tropical richness;
(3) region of long-term humid conditions (Pleistocene refugia);
(4) remote and poorly explored;
(5) semi-isolated and archipelago-like habitat islands;
(6) relatively small size of habitat islands;
(7) high levels of endemism in other groups.

North Annam fulfills all these criteria admirably.

Most of the localized endemic vertebrates of Asia show montane or insular distributions: Western Ghats, southeast China mountains, central China mountains, Mt. Kerinci (Sumatra), Mt. Kinabalu (Borneo), Taiwan, Mt. Victoria (Burma), West Javan mountains, Sulawesi, the Philippines, and the Mollucan islands. Contrast these with the generalized Asian large mammal fauna of elephant (*Elephas maximus*), tiger (*Panthera tigris*), leopard (*Panthera pardus*), gaur (*Bos gaurus*), wild boar (*Sus scrofa*), sambar deer (*Cervus unicolor*), and red muntjac (*Muntiacus muntjak*), which occur broadly from northwest India to Borneo, and through a huge range of altitudinal and rainfall differences. These latter large mammals probably constitute a fauna that has followed Man south and east through Asia, benefiting from human opening and burning of the forests and displacing the original, more evergreen-forest fauna. Glimpses of this richer fauna can be seen in the fossil record of the Siwaliks of north India.

In Africa, one also finds that endemism and species richness are concentrated around relict evergreen mountains and Pleistocene refugia: west African rainforest, Mt. Cameroon, eastern Rift forests, and east Tanzanian forests and mountains. In contrast, the huge forests of the Congo basin and the huge savanna plains of east and southern Africa have little endemism.

The new mammals of the 21st century will be found in the still unexplored regions that meet the criteria mentioned above of isolation, tending to be tropical and evergreen systems, and lying within the regions of high species diversity or endemism. Such unexplored areas remain in northeast India, Burma, Laos, southeast Tibet, northwest Yunnan, south Philippines, New Guinea, peripheral mountains of the Amazon Basin, isolated mountains of Central America, as well as smaller neotropical drainage systems.

In addition, there are many new descriptions to be made among lesser explored taxa and the less spectacular and more difficult small mammals. Birds are much better known than mammals because they are mostly diurnal, can mostly be recognized in the wild at long range or by vocalization, and because the world is swarming with rather professional amateur birdwatchers. In contrast, most small mammals are nocturnal, live in concealed spots, and are very difficult to identify. And sometimes they smell and bite!

However, the scientist now has several important new tools to help predict where species are likely to be and also to record, catch, and distinguish species. Satellite imagery allows habitats to be more easily recognized and mapped, which enables potential species distributions to be determined. Automatic cameras allow shy secretive animals to be recorded. Tape recorders and sonographs allow vocalizations to be used for identification and to distinguish between forms. GPS (geographical positioning systems) allow much greater accuracy in locality information, which enables a tighter definition of species habitat requirements. IT (information technology) allows much faster comparison of material by scientists. Air travel and better communications allow biologists access to areas previously only accessible by major expedition. DNA analysis provides a whole new high-resolution technique for discriminating relationships between populations and species.

For instance, the common Grant's gazelle (*Gazella granti*) of East Africa resolves into at least three different species on the basis of DNA differences (P. Arctander, pers. comm.). The whole concept of what is a species is raised once again. Some species seem morphologically very distinct, but DNA reveals they are not. Other species are morphologically inseparable but found to be very different genetically. Efforts to define objective morphological criteria for defining species start to break down. Whether one adopts a biological species concept or a phylogenetic species concept, it is expected that good species in nature maintain discrete breeding. However, more and more evidence of cross-breeding between wild species is discovered. Most organisms accept alternatives if the perfect mate is not available, and breeding among closely related species does result in hybridization. All members of the family Cervidae can be made to hybridize with all other members in captivity (Arctander, pers. comm.). However, even wide, stable hybrid zones can be accepted without invalidating species status if hybrids remain at a disadvantage in the mating game. With birds, for instance, it can usually be demonstrated by playback experiments that there is a stronger response

to the true species call than to either the call of the second species or a hybrid. As more of these species come under DNA scrutiny we will be able to move closer to a phylogenetic species concept, and many forms now placed conservatively within one species will split.

The question of where species begin and end becomes more complicated when legal aspects become involved for protection, control of trade, and ownership of genetically modified organisms. The least recognized unit is ultimately the individual. Species evolve from one into another and split gradually from one to two through isolation. There is no objective cut-off point. We must accept that our criteria are rather subjective and not consistent across the board. We must redefine satisfactory new ways to label the individual. The question of "How many species are there?" returns again to the age-old taxonomists' puzzle of "What is a species?"

The world loses species and genetic variety at an unprecedented pace, but thanks to taxonomists, our lists grow longer and longer and we should be on guard lest a sense of loss is dulled by new discovery. It is important that taxonomists become aligned to the conservation movement, where their skills are sorely needed. Monitoring biodiversity is as important as describing biodiversity.

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THE TROPICAL FLORA REMAINS UNDERCOLLECTED[1]

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ABSTRACT

Recent fieldwork of the Royal Botanic Gardens, Kew, in many parts of the tropics reveals the extent to which they are still undercollected and poorly studied. Recent studies of palms in Madagascar, Cameroon, Lao P.D.R., and Brunei Darussalam have produced many novelties, for example, in Madagascar, 3 new genera and 85 new species. Recent examples from Atlantic coastal Brazil, central Amazonia, and New Guinea are given. Even in apparently well collected areas such as the Ducke Forest Reserve near Manaus, Brazil, and in Brunei where detailed studies of small areas are made, many novelties are found. It is recommended that more such intensive studies of restricted areas are made. The rate of new species that are being described, an average of 2350 over the past nine years, and the rate of additions to *Flora Neotropica* suggest that the total number of angiosperms is currently being underestimated and that there are in fact between 300,000 and 320,000 species. In order to develop conservation and sustainable use of tropical ecosystems, it is essential that we continue to intensify the rate of collection before it is too late.

Key words: Amazonia, biodiversity, Brazil, Brunei Darussalam, Cameroon, conservation, field inventory, Lao P.D.R., Madagascar, New Guinea, palms, tropics.

The purpose of this paper is to show, with examples from a few places, that the tropical flora remains severely undercollected and that many new species of angiosperms continue to be discovered and described every year. The destruction of tropical habitats both in areas of rainforest and in savannas continues at an alarming rate and this before we have completed the inventory of what exists. As a result of the incomplete inventory, we seem to be underestimating the total number of seed plants in the world. We have identified a few areas of the world where further collecting is urgent and destruction of vegetation is continuing at alarming rates from a variety of causes. It is our hope that this will encourage greater efforts in both collection of material and habitat conservation before it is too late. The areas described are places in which the Royal Botanic Gardens, Kew, is involved, and they serve as examples of what is also occurring in many other places and in many different ecosystems, both tropical and temperate.

THE MATA ATLANTICA OF BRAZIL

We commence with this area because its destruction is now so well documented and only about 6% of the Atlantic coastal rainforest of Brazil remains intact. It has been devastated mainly by the establishment of cacao plantations and sugar cane fields as well as by other types of farming. Many studies have shown the high level of endemism in this area. For example, Mori et al. (1981) showed from a sample of 127 trees described in *Flora Neotropica* monographs that 53.5% were endemic to the Mata Atlantica. There is no doubt that some of these locally described species are now extinct. However, recent collecting in the remaining fragments of this ecosystem continues to turn up many new species and also interesting disjunct distributions. Two examples of this are *Brodriguesia santosii* R. S. Cowan and the new genus *Harleyodendron*, with its single species *H. unifoliatum* R. S. Cowan (Cowan, 1979, 1981). An interesting range extension is the recent first collection of *Anthodiscus* (Caryocaraceae) in the Mata Atlantica, a genus that was previously only known from the Guianas, western Amazonia, and the Chocó in Colombia. *Anthodiscus amazonicus* Gleason was found in the forests of Bahia (Prance & Mori, 1980) and added yet another Amazonian disjunct to the many cited by Lima (1953). If the remnants of the Mata Atlantica are yielding so many novelties, what have we lost in the 94% of the region that has been deforested? The conservation of the remnants, now declared a World Heritage site, is vital for the future reconstruction of this habitat.

[1] We thank the many local institutions and collaborators who have assisted in the fieldwork in the areas discussed here. GTP thanks the Instituto Nacional de Pesquisas da Amazona, Michael Hopkins, and all the Reserva Ducke team for their help with the project from which data is cited.

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Table 1. New species found in the 100 km² Reserva Florestal Adolpho Ducke, Manaus, Brazil in the course of preparing a flora.

Family	No. of species
Lauraceae	11
Sapotaceae	10
Annonaceae	6
Araceae	6
Arecaceae	3
Lecythidaceae	3
Passifloraceae	3
Chrysobalanaceae	2
Rubiaceae	2
Other families	9
Total	55

The Reserva Florestal Adolfo Ducke, Amazonas, Brazil

This 100-km² forest reserve near Manaus in central Amazonia was believed to be one of the best-collected areas in Amazonia. In 1993, we set out to prepare a Flora and a field guide to the reserve and initiated intensive collecting. The preliminary list from the INPA herbarium database contained 825 species (Prance, 1990), and so we assumed we were working with a flora of about 1000 species. Five years later we now have 2175 species to be included in the Flora (M. Hopkins, pers. comm.). This includes over 50 new species (see Table 1). This work shows the value of the intensive study of a small area of tropical forest and the value of florulas. The recently published florula of the forest reserves around Iquitos, Peru, published by the Missouri Botanical Garden, is another good example of a local flora that has helped to improve the stocks of the botanical inventory of an area (Vásquez Martínez, 1997). In order to complete the inventory of the tropics, we need many more of these detailed floristic studies of small areas.

Madagascar

Madagascar is another place that is notable both for its high level of endemism of plants and animals and for the rapid destruction of the natural vegetation. It is also badly undercollected despite the recent efforts of both the Missouri and Kew gardens. The latter has made a recent study of the palms, which serve as an example of what remains to be done and what can be found when specialists concentrate on a particular group of plants.

Like many tropical countries with a half-finished flora, the families that have been treated in such floras are incomplete. The Palmae were written in 1945 for the *Flore de Madagascar et des Comores* by Jumelle and Perrier de la Bâthie, acknowledged experts on palm taxonomy. The volume described and keyed 115 species, but descriptions were often based on scrappy specimens and therefore the keys failed to work for most taxa. Consequently, palm information for Madagascar was confused, and this for a family in which many ethnobotanical uses were recorded: palms are used for food, construction, basketry, medicine, and many other items in Madagascar.

When Uhl and Dransfield (1987) published their monumental *Genera Palmarum*, their main problems with generic delimitation and tribal relationships were in Madagascar. Therefore Dransfield embarked on a series of field trips to the island, but soon started discovering so many new species and additional mysteries that he decided a special Madagascar project was needed. With funding from McDonald's Restaurants (UK) the project was set up, and Henk Beentje started working for it in 1991. He lived in Madagascar for a year and a half, traveling all over the east coast in search of palms.

The results of this fieldwork plus further visits by Dransfield showed how much we did not know: 3 new genera and 85 new species were discovered (Dransfield & Beentje, 1995). These included the world's smallest palm (*Dypsis tenuissima* Beentje, less than a foot high) but also 80-feet-high canopy palms such as *Voanioala*, *Lemurophoenix*, and *Orania ravaka* Beentje, and an amazing aquatic palm. The latter, *Ravenea musicalis* Beentje, has seeds that float on the water. A small bump splits the fleshy fruit wall, and releases the seed, which then sinks. The seed has already developed a half-inch-long hooked seed leaf within the fruit (a strategy analogous to several mangroves), and this hook catches on projections on the streambed, enabling the seedling to start rooting. The worrying thing is that this palm only seems to grow in a single river, in which 450 trees have been counted. Many of the newly discovered palms have very restricted distribution, and coupled to the threats to the vegetation where they occur, this emphasizes two things: the need to conserve, and the need to inventory such dwindling habitats.

Yet another new genus, *Satranala*, was known from a few tens of trees in a single reserve; on a recent trip, Dransfield visited a second newly discovered site, bringing the known numbers to the low hundreds. Why are such palms so rare? In the

case of *Satranala*, there is an intriguing hypothesis. The fruit endocarp is hard and shows strong flanges, unlike any other palm endocarp—apart from a few taxa from New Guinea, distributed by cassowaries. The hypothesis is that *Satranala* was distributed by the giant elephant bird, the roc of the Thousand and One Nights, the *Aepyornis* of science: a bird now extinct, though its subfossil eggs are still found on Madagascar. Therefore, because *Aepyornis* is extinct, long-distance dispersal of *Satranala* does not take place any more, and its area of distribution dwindles.

If the palm project had started 10 years later, several of the new species would not have been discovered, as they would have become extinct in the meantime. With information from projects such as these, focused conservation can take place next to general conservation, and save species. Without the inventory being completed, these rare species will just go extinct.

The Palms of Cameroon, Lao P.D.R., and Brunei Darussalam

Palms are one of the groups of plants that one might expect to be well known and well collected because they are often both large conspicuous plants and almost all are used in some way by local peoples. On the other hand, non-specialist collectors have tended either to avoid collecting palms or to make poor specimens because of the special difficulties of collecting such large-leaved and often spiny plants. A brief summary of three recent field projects involving John Dransfield shows what still remains to be done in the field to get even a basic catalogue of palms.

CAMEROON

With relatively few species (about 20 in all), the continent of Africa has a poor rattan flora when compared with Southeast Asia (Uhl & Dransfield, 1987). The rattans that do occur are certainly conspicuous and are sometimes visible in great thickets along roads in perhumid areas of equatorial Africa. They remain poorly understood, largely because they have rarely been adequately collected. For some time it has been assumed that in the genus *Laccosperma* (ca. 5 species) there was one large-diameter species, *Laccosperma secundiflorum* (P. Beauv.) Kuntze. Beccari had described a second large species, *L. acutiflorum* (Becc.) J. Dransfield (as *Ancistrophyllum acutiflorum* Becc.), based on very poor material. Subsequent workers have been content to include the latter as a synonym with *L. secundiflorum*. During fieldwork in December 1997 near Kribi in Cameroon, Terry Sunderland and John Dransfield found extensive populations along the main road of two clearly distinct, easily separable large-diameter rattans that match the two named taxa. They found it astonishing that two taxa so clearly distinct, could have been confused; no doubt the difficulty of collecting these spiny plants was responsible for their poor representation in herbaria. Terry Sunderland is now conducting a critical survey of all African rattans to put their taxonomy and economic development onto a firm basis.

LAO PEOPLES' DEMOCRATIC REPUBLIC

Lao represents one of the largest gaps in our knowledge of palms. Until two years ago, only three palms were recorded in the literature as occurring in Lao P.D.R. As a result of a preliminary survey of rattans by the Lao Forestry Department, funded by the International Development Research Centre and the International Network for Bamboo and Rattan, we know that there are at least 30 species of rattan in Lao P.D.R., but these have yet to be critically named. In order to provide a firm base for future rattan development in the country, the Lao Forest Department, together with Oxford Forestry Institute and Royal Botanic Gardens, Kew, has started a new critical survey of Lao rattans and ecological work aimed at understanding the demography of Lao rattans. This is funded by the UK Darwin Initiative. One of the first and most surprising results of the survey is that the premier large-diameter cane that is being harvested in huge quantities in Lao, much of it being shipped over the border to Vietnam, appears to be an undescribed taxon.

BRUNEI DARUSSALAM

Until the start in 1988 of Kew's project to prepare a checklist of the plants of Brunei, there were a mere 17 palms recorded for the country. Yet this small nation has one of the highest collecting indices for the whole of the *Flora Malesiana* region—just the sort of statistic that added to the difficulty in justifying further intensive work in the country. However, as in the case of the Reserva Ducke, intensive fieldwork in a relatively well collected area by specialists always yields results. By the end of the project in 1995 the palm list had risen from 17 to 140 different species, showing that almost half the Bornean palm flora occurs within the well protected forests of Brunei. During fieldwork several unusual new taxa were unearthed, including the smallest species of *Livistona*, *L. exigua* J. Dransfield, the handsome *Pinanga yassinii* J. Dransfield,

Table 2. Data from *Index Kewensis* on number of taxa.

Year	Genera		Species		Subspecific taxa subspecies and variety	
	New taxa only	All incl. new combinations & new names	New taxa only	All incl. new combinations & new names	New taxa only	All incl. new combinations & new names
1989			2752			
1990			2653			
1991			2220			
1992			1705			
1993	77	88	2049	3436	508	1663
1994	92	102	2126	3557	517	1398
1995	107	118	2413	4053	487	1400
1996	95	106	2409	4168	467	1493
1997	79	94	2770	4342	543	1586
5-Year total	450	508	11,767	19,556	2522	7540
9-Year total			21,097			

and, among the non-palms, the extraordinary *Orchidantha holttumii* K. Larsen (Lowiaceae).

New Guinea

Of all the places in the tropics, New Guinea probably remains the least known. This is certainly evident from the results of recent fieldwork organized by Kew in Irian Jaya, where a large number of the collections turn out to be new species. In Irian Jaya, collecting density estimates show that less than 25 specimens have been collected per 100 km^2, and there are only slightly under 140,000 collections from a territory with an estimated flora of 25,000 species. The neighboring Papua New Guinea has a collection index of well under 50 specimens per 100 km^2 and is also poorly known. New Guinea offers a particularly large range of habitats because of its rugged topography, where you can go from coastal mangrove to the glacier on Mount Jaya in a distance of only 110 km. The flora varies from tropical rainforest to high alpine. New Guinea is a center of diversity for many important tropical groups such as tree ferns (*Cyathea* and *Dicksonia*), Pandanaceae (a recently collected new species has thick fleshy, fruity smelling bracts that are eaten by bats, which are probably its pollinators), and Myristicaceae (for example, the recently described nutmeg species *Myristica inaequalis* W. J. de Wilde).

Fieldwork in Irian Jaya is also turning up many interesting new records such as the recent collection of *Ternstroemia magnifica* Stapf ex Ridley, which was thought to be distributed from the Malay Peninsula to Sulawesi. However, it has now been shown to occur in Irian Jaya.

The importance of collecting these basic data about the plants of New Guinea has been emphasized by the fact that Robert Johns has been able to prepare maps of proposed conservation areas for both Irian Jaya and Papua New Guinea. These maps are based largely on plant and vegetation data assembled as the result of Johns's collecting activities. This application to systematic data is a vital activity in an area where it is not too late to conserve large tracts of this highly endemic flora, which is now under severe pressure from both mining and timber extraction.

How Many Species to Go?

The most recent estimate of the number of species of angiosperms in the world is 250,000 and was originally based on a calculation by Stebbins (1974) and confirmed or cited by many other authors, including myself (Prance, 1977; Groombridge, 1992). These calculations were based on a family-by-family listing of species and provided a good estimate based on data of the 1970s. However, I am sure that this estimate must now be raised considerably. Data in Table 2 show the descriptive activity of botanists between 1989 and 1997, during which period 21,097 new species were described. Furthermore, the annual rate has not declined over the nine years involved. This is backed up by data from other sources: for example, 29% of the species treated in *Flora Neotropica* monographs are new (Wm. Wayte Thomas, pers. comm.).

These data show two interesting points. Firstly, there is obviously still a lot more collecting to be done, if an average of about 2350 species are being added each year; and secondly, despite the many claims that descriptive taxonomy is on the decline, we are still describing species at the same rate.

The fact that we have added 21,097 species over nine years, and also the comparison between 1970 and 1997 data for a few selected plant families, leads us to conclude that there are actually between 300,000 and 320,000 species of angiosperms.

Conclusion

Data from both the undercollected areas described here and the study of the rate of description of new taxa clearly demonstrate that the field inventory of the angiosperms is far from complete. It is necessary to continue to invest considerable resources into fieldwork and descriptive taxonomy and not be tempted to divert them all to the equally important and exciting new techniques of molecular systematics. More detailed studies of selected small areas in the tropics are likely to yield many more new species as well as most useful demographic data about those that are already described. The more complete the inventory is the better the data we will have to provide the rationale for conservation and for the sustainable use of ecosystems.

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WHAT IS SIGNIFICANT—THE WOLLEMI PINE OR THE SOUTHERN RUSHES?[1]

Barbara G. Briggs[2]

Abstract

The discovery in 1994 of *Wollemia nobilis*, a new conifer genus and species of Araucariaceae, attracted media and public attention that was probably unprecedented for a botanical discovery. If a plant species may be called charismatic, it is this one: tall, handsome, rare, of a lineage dating from the Jurassic, surviving undiscovered in a mountain gorge. It was front-page news around the world and soon became one of Australia's most publicized species. Scientists shared the enthusiasm, offering research collaboration to investigate its many aspects. *Wollemia* has contributed to understanding of structures in fossil Araucariaceae and conifer-mycorrhizal associations; its survival has added to the picture of long-term regional floristic change. After an extended period of small population size it shows no detectable inter-plant genetic diversity—relevant to the management of rare plant species. Its discovery helped us explain and emphasize to the community the value and nature of biological research and the need for habitat conservation. At the opposite end of the charisma scale are the southern rushes, Australia's relatively inconspicuous Restionaceae and their allies (Centrolepidaceae, Ecdeiocoleaceae, Anarthriaceae). Despite their links with other southern continents and close relationship to the Poaceae, these had been greatly neglected for over 100 years and were largely misclassified generically. Study and fieldwork in recent decades have revealed 61 formerly undescribed species, nearly 40% of the total now distinguished in these families for Australia. DNA sequencing of plastid genes gave surprising results, with evidence that two new plant families should be recognized. New findings are contributing to better understanding of the ancestry of related families and Southern Hemisphere floras. Distinguishing the new rare species allows focus on their conservation needs. Many new species are still being recognized in Australia's flora, among flowering plants and conifers as well as other groups. *Wollemia* and the southern rushes exemplify the significance of these new finds and newly discovered understanding of relationships. In each case the significance of the discoveries is realized only in the context of the knowledge of organisms and their evolution that comes from research in many fields.

Key words: Araucariaceae, Australia, conservation, Restionaceae, Southern Hemisphere floras, *Wollemia*.

Wollemia—A Charismatic Subject for Research

In 1994 my colleagues and I at the National Herbarium of New South Wales in Sydney realized that we had been given a remarkable opportunity—the discovery of a new plant species that would catch public and scientific attention in a truly outstanding way.

David Noble of the New South Wales National Parks and Wildlife Service had found about 40 trees of a previously unknown species, soon to be named *Wollemia nobilis* W. G. Jones, K. D. Hill & J. M. Allen, a new member of the conifer family Araucariaceae (Jones et al., 1995). This rare and highly restricted species had been discovered about 200 km northwest of Sydney, in a deep gorge bounded by sandstone cliffs (McGhee, 1995; Duffy, 1997). Such a site would differ from most of the surrounding area in the constancy of water supply, more equable climate, and especially in being largely protected from wildfire, although one tree showed evidence of fire, followed by resprouting. The trees are mostly emergents overtopping both a dense fern layer and a canopy of closed forest (warm temperate rainforest) of *Doryphora sassafras* Endl. and *Ceratopetalum apetalum* D. Don. Seedlings are present (about 200 juveniles were recorded; Nash, 1997; Offord et al., 1999), but most only produce a few leaves, failing to grow to maturity unless a break occurs in the canopy.

The fossil record of the Araucariaceae has been studied extensively. The family appeared in the late Triassic, with a peak of diversity in the Jurassic and a continued decline since the end of the Cretaceous (Miller, 1977). Some of the earliest Araucariaceae are reported from the Northern Hemisphere, and fossil pollen with *Araucaria*-like features is widespread in both hemispheres in the Jurassic and Cretaceous. Its present survival in the south is thus relictual, rather than implying a Gondwanic origin

[1] I thank my colleagues John Benson, Sue Bullock, Ken Hill, Adam Marchant, Patricia Meagher, Cathy Offord, Carolyn Porter, and Brett Summerell for discussions and for making available unpublished findings. Deborah McGerty assisted with the illustrations, while Neville Marchant, Director of the PERTH herbarium, and Bruce Fuhrer gave permission to use photographs.

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Figure 1. *Wollemia nobilis* in its natural habitat. Photo Jamie Plaza.

Figure 2. —A (top). Foliage of *Wollemia nobilis* with *Podozamites* (also known as *Agathis jurassica*). This Jurassic fossil conifer is probably not the closest relative of *Wollemia*, but is similar especially to *Wollemia*'s juvenile foliage. —B (bottom). Cone scales of *Wollemia* and of a Jurassic fossil member of the Araucariaceae. Photos J. Plaza.

(Gilmore & Hill, 1997; Setoguchi et al., 1998). Before the discovery of *Wollemia*, the family was known from South America, New Zealand, northeastern Australia, New Guinea, and, in especially rich diversity, in New Caledonia. Two extant genera had been recognized, *Araucaria* and *Agathis*.

The newly discovered *Wollemia* created a sensation. It was handsome, and so large—up to 40 m tall—that it was almost unbelievable that it had been unknown to science until now (Fig. 1). It linked with fossils that connected to ancient groups, back to the Jurassic (Fig. 2), and this in an age when dinosaurs have an unrivalled fascination for adults as well as children. Its habitat, in deep mountain ravines, held an almost sinister appeal.

What followed would not apply to the average newly found species.

A media conference was called to announce the discovery. This brought a response that exceeded our expectations: it was briefly front-page news in the press around the world, with journalists and science writers seeking further information. Television programs and tapes were prepared, featuring its discovery and the subsequent research.

An interagency government committee was set up to develop a conservation plan (Nash, 1997) and to monitor threats and actions affecting its survival. Strict protocols for visits to the habitat were established, especially changing shoes at entry to the site, to avoid bringing in pathogens. Approvals to visit were highly restricted.

Milton Silverman, who had gone from San Francisco with Ralph Chaney in 1948 to collect the dawn redwood (*Metasequoia glyptostroboides* Hu & W. C. Cheng) in western China, wrote to congratulate us on our efforts and the discovery, which was almost ironic considering how much more accessible our find had been than theirs. The name "Wollemi pine" was coined so that we would not seem too lacking in words in reporting the discovery of a plant that had not yet been botanically named. The intrepid travelers to China had coined "dawn redwood" partly because *Metasequoia glyptostroboides* would not fit across a newspaper column in reporting their collections of that species,

which, shortly before, had been discovered and compared with fossil species.

Horticultural research and propagation started immediately, using cuttings and seeds (Fensom & Offord, 1998; Offord et al., 1999). The aims were to learn the propagation requirements, establish a conservation population in cultivation, and eventually to safeguard the species by widespread use in horticulture. As in other Araucariaceae, growth was found to be plagiotropic, with plants raised from cuttings of lateral branches mostly continuing to grow horizontally, whereas those from erect stems continue erect growth. Advertisements for commercial partners in raising large numbers of plants brought many proposals. When a young tree was planted in the Royal Botanic Gardens in Sydney, this was done with ceremony by a senior government member, and the plant was enclosed in a very stout cage.

Offers to collaborate in a diversity of research approaches flowed in, 30 within two weeks of announcing the discovery on the TAXACOM listserver (Brooks, 1997). Studies of genetics, chemical constituents, embryology, and anatomy, as well as associated fungi and insects were soon focused on *Wollemia*. Sequencing of the plastid gene *rbc*L (Gilmore & Hill, 1997) confirmed the distinctness of *Wollemia*, although different analyses using different ranges of other taxa gave sharply contrasting phylogenies for the Araucariaceae. Gilmore and Hill (1997) and Stefanovíc et al. (1998) found *Wollemia* to be sister to *Agathis*, with those two genera forming a clade that is sister to *Araucaria*. By contrast, Setoguchi et al. (1998), using the same sequence data for *Wollemia* but combined with a different range of other conifer taxa, concluded that *Wollemia* diverged before the separation of *Araucaria* and *Agathis*. It is hoped that the study of other genes will resolve this discrepancy.

Comparison of *Wollemia*'s adult and juvenile foliage, stomates, pollen, and cone scales with other living and fossil Araucariaceae (Chambers et al., 1998) helped in the interpretation of fossil Araucariaceae, especially in the structure of the cone scales and seed. Its tree architecture was described as unique (Hill, 1997), differing from previously described structural models and other Araucariaceae. Male and female cones are each terminal on a first-order, short-lived lateral branch, and coppicing is a consistent feature. Leaf anatomy has been studied (Burrows & Bullock, 1999) and so has reproduction (Offord et al., 1999). The pollen was found to be indistinguishable from the fossil pollen form-genus *Dillwinites*, which is recorded in Australia and New Zealand extending back to the late Cretaceous (Macphail et al., 1995; Chambers et al., 1998). The most recent fossil records of this pollen type, from Bass Strait, are about 2 million years old.

An endophytic fungus, *Pestalotiopsis guepinii*, was isolated from *Wollemia* (Strobel et al., 1997) and found to produce taxol, which has anti-cancer properties and is effective in controlling oomycetous fungi. The mycorrhizal associates and susceptibility to common pathogens were studied (B. Summerell, pers. comm.). More than 50 taxa of fungi have been recovered from the trees and their immediate surroundings in a survey that cultured fungi from seeds, leaves, leaf litter, roots, root debris, and soil. Such a number of fungal associates is thought to be typical for a tree species in such an environment, but comparisons are uncertain since few tree species have been examined so comprehensively. Of these 50 fungal taxa, 9 are thought to be undescribed species. Some roots are densely filled with endophytic fungi whereas ectophytic fungi are found in other cases, an unusual condition observed also in other conifers (McGee et al., 1999).

Other studies have focused on *Wollemia* especially because it is so rare and vulnerable (Benson, 1996; Offord, 1996). *Wollemia* was found to be susceptible to some pathogens, including *Phytophthora cinnamomi*, which has been introduced and is spreading in southeast and southwest Australia, confirming the need for strict protocols for site visits. Its population genetics was investigated with studies of allozymes and DNA (Peakall, 1998), and these showed no discernible genetic variation among individuals, although more than 800 loci were evaluated with AFLP fingerprinting. There may be some clonal spread, since plants coppice and some have multiple trunks, but the genetic findings are interpreted as largely the result of extremely low population size over a long time, an extended genetic bottleneck. Preliminary data on *Agathis* and *Araucaria*, while showing some variation, indicate that genetic variability is low in the family as a whole (Peakall, 1998).

Was the concentration of attention on *Wollemia* in the media and from scientists justified? This question resonated especially when answering questions about the scientific significance of the find, while in my view the slender silhouettes of other members of the Araucariaceae towered over trees of lesser stature on the skyline of Sydney's Royal Botanic Gardens. That question will be considered after reviewing a contrasting example.

The Southern Rushes

The Restionaceae and allied families in Australia, the southern rushes, are as much in need of charisma as *Wollemia* is blessed with it. The flowers are inconspicuous, with small scarious bracts, glumes, and tepals (Fig. 3) and with the leaves reduced to sheathing scales (Meney & Pate, 1999). They occur exclusively in low-fertility soils and in arid or seasonally waterlogged sites, habitats avoided for agriculture and therefore of low human population and often poor access. In one of the allied families, Centrolepidaceae, the plants are tiny, some species no more than 1 cm tall.

Through such features Restionaceae seem to have brought on themselves extreme botanical neglect over the first half of this century. Before then, the three great early figures of Australian botany had made a good start on their discovery and classification. Robert Brown, naturalist on the first circumnavigation of Australia in 1801–1803 described seven genera and 47 species of Restionaceae that are currently recognized (Brown, 1810). A further 10 species were named by Ferdinand Mueller (1873), whereas George Bentham (1878) recognized 71 species. By the early 1960s, when L. A. S. Johnson and I started our investigations, the number of known species had crept to 86. Around that time also David Cutler of the Royal Botanic Gardens, Kew, made extensive anatomical investigations (Cutler, 1969). It became clear that the generic classification was entirely inadequate (Cutler, 1969, 1972; Johnson & Briggs, 1981; Briggs & Johnson, 1998a) and that many specimens matched no named species.

Clearly this neglected plant group would present a fertile field for new discoveries, but it far exceeded expectations. The discoveries have been both new species and new understanding of relationships, necessitating a radical reclassification.

In recent decades, great swathes of country had become more accessible, especially in the sandplains of Western Australia. Examining the unidentified collections in herbaria revealed many new species; fieldwork brought additional ones. Even the largest of all Australian restiads, with flowering stems over 2 m tall, is among the recently discovered species yet to be formally named. Investigation of supposedly variable species often showed these to be assemblages of several allied species, each with a distinctive distribution and ecological range. The study brought to light 51 new species, mostly from the south of Western Australia. Just when we thought that few additional finds could be expected in Australian Restionaceae, colleagues in Western Australia found a further 10 species (Meney et al., 1996). There was notably little hybridization among the species; the distinctions were sometimes inconspicuous, but they were consistent.

Restionaceae show an exceptionally high proportion of newly recognized species, but it is estimated that about 15% of Australia's flowering plants are still to be discovered (A. Orchard, pers. comm.), in addition to many now distinguished but awaiting publication.

The new view of Australian Restionaceae did not stop at species. When this study began, 29 Australian species were named within the genus *Restio*, but it became clear that *Restio* was a member of a group of genera limited to Africa and Madagascar (Cutler, 1972; Johnson & Briggs, 1981; Linder, 1985, 1991; Briggs & Johnson, 1999). Therefore, new genera were required, or old synonyms taken into use, to accommodate all the Australian species hitherto included there (Briggs & Johnson, 1998a, b).

Moreover, Restionaceae showed a pattern similar to that in Proteaceae, Fabaceae, Ericaceae, and Poaceae in their post-Gondwanic floristic richness. The history of climates, migrations, and survivals has left different traces on the African and Australian continents, so that Africa has large numbers of species in relatively few genera, but Australia has a diversity of groups appropriate for recognition as genera. This pattern has shown even when the same botanist studied groups in both continents, rather than being an artifact of different generic concepts (Peter Weston, pers. comm.; Nigel Barker, pers. comm.), although it is not apparent when Proteaceae of the Cape Region are compared with only the southwest of Western Australia (Cowling & Lamont, 1998). Our case led to the description of the rather alarming number of 16 new genera of non-African Restionaceae (Briggs & Johnson, 1998a).

Morphological cladistics (Linder et al., 2000) and DNA data both indicate that an early division within Restionaceae is between the African clade and the Australasian clade (though the DNA data give only weak support). This would be consistent with an ancient Gondwanic connection. By contrast, the single species in South America, *Apodasmia chilensis* (Gay) B. G. Briggs & L. A. S. Johnson, is extremely similar to the New Zealand *A. similis* (Edgar) B. G. Briggs & L. A. S. Johnson, indicating long-distance dispersal. Moreover, *Apodasmia* (recently segregated from *Leptocarpus;* Briggs & Johnson, 1998a) includes foredune coastal species and is the only notably salt-tolerant genus of the family; it is singularly well suited to establish successfully after dispersal.

Sequencing of plastid DNA was done in parallel with morphological studies and gave a further unexpected result, evidence that two new plant families should be recognized (Briggs et al., 2000). *Hopkinsia* and *Lyginia* are small genera, of two and three species, respectively (one species of each genus undescribed). Their inclusion in Restionaceae had never been questioned, even when they were the subject of detailed anatomical investigations (Gilg, 1890; Cutler, 1969). But two sets of DNA sequence data (from *rbc*L, and from the *trn*L intron with the *trn*L-*trn*F spacer) are consistent in grouping them (each with 100% jackknife support) with *Anarthria* (Fig. 4) rather than with Restionaceae. That grouping is shown in a jackknife consensus tree from analysis of the total sequence from these DNA regions and is further supported by the presence of two distinctive indels (one insertion and one deletion) in the *trn*L intron (Fig. 4).

Although further investigations are needed and proceeding, it appears that the *Anarthria* clade (*Anarthria, Hopkinsia, Lyginia*) is not the sister group to Restionaceae. That position appears to be held by Centrolepidaceae. Such close affinities, or even inclusion of Centrolepidaceae within Restionaceae, have been suggested on morphological and embryological grounds (Hamann, 1962, 1975; Kellogg & Linder, 1995; Linder et al., 2000) and now have some support (although not robust) from analyses of DNA data (Fig. 4; Briggs et al., 2000). It has been suggested that Centrolepidaceae are neotenous, with mature plants showing some similarities to seedlings of related families, although their inflorescences are very different.

Despite the evidence that *Hopkinsia, Lyginia,* and *Anarthria* form a clade, they share no synapomorphies of morphology, anatomy, flavonoids, pollen, or seeds, except for features that are either plesiomorphic within the Poales or widespread in the order. Similarly, studying these genera in light of the DNA data showed that those features that they have in common with Restionaceae are plesiomorphies, although each has distinctive autapomorphies. *Hopkinsia* has a reduced carpel number and succulent indehiscent fruits. *Lyginia* shows a distinctive arrangement of thick- and thin-walled cells interspersed in the chlorenchyma, sloping stomates, fused stamen filaments, and highly distinctive seeds ornamented by minute pits and spines. *Anarthria* lacks a sclerenchyma cylinder in the culms and has unreduced, ensiform leaves and also large chromosomes (Briggs, 1966). Such chromosomes are, in general, associated with large genomes, an apomorphic feature (Bennetzen & Kellogg, 1997; Bennett & Leitch, 2000). Without some morphological basis there appears to be no case for enlarging Anarthriaceae or describing a single new family for *Hopkinsia* and *Lyginia.* The most logical course is the recognition of "Hopkinsiaceae" and "Lyginiaceae," and these new families are being described (Briggs & Johnson, in press).

The families mentioned above, Restionaceae, Centrolepidaceae, Anarthriaceae, "Hopkinsiaceae," "Lyginiaceae," together with the Ecdeiocoleaceae, Joinvilleaceae, and Flagellariaceae, appear to be the closest relatives of the Poaceae (Dahlgren et al., 1985; Chase et al., 1993; Duvall et al., 1993; Briggs et al., 2000). These are all families with primarily Southern Hemisphere distribution. Especially notable is their high concentration in the southwest of the Australian continent: six of the eight families occur in that region and four are limited to it. Together with nine other families they constitute the Poales as recognized by the Angiosperm Phylogeny Group (APG, 1998). Three of the other families are primarily in the Southern Hemisphere (Hydatellaceae, placed here but with little evidence, Prioniaceae, and Thurniaceae), while six are distributed in both hemispheres (Cyperaceae, Eriocaulaceae, Juncaceae, Sparganiaceae, Typhaceae, and Xyridaceae). The concentration of allied families in the south has led to the suggestion that the Poaceae itself had a southern origin (Doyle et al., 1992), although these distributions could also be relictual, as with the Araucariaceae.

What Is Significant?

The examples considered above are two ends of a spectrum. In *Wollemia nobilis* we have a single new species and genus in a recognized plant family, but an exceptionally charismatic and interesting find. In Restionaceae and its allies we have some 60 new species, many new genera, and two new

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Figure 3. Australian Restionaceae and Anarthriaceae. —A (top left). *Lepidobolus preissianus*, male (left) and female spikelets, each ca. 1.5 cm long. —B (top right). *Baloskion tetraphyllum* subsp. *meiostachyum*, female, spikelets ca. 5 mm long. —C (bottom left). *Meeboldina scariosa*, male, spikelets ca. 4 mm long; photo B. Fuhrer. —D (bottom right). *Anarthria scabra*, female, with prominent stigmas; linear leaves ca. 6 mm wide; photo B. Fuhrer.

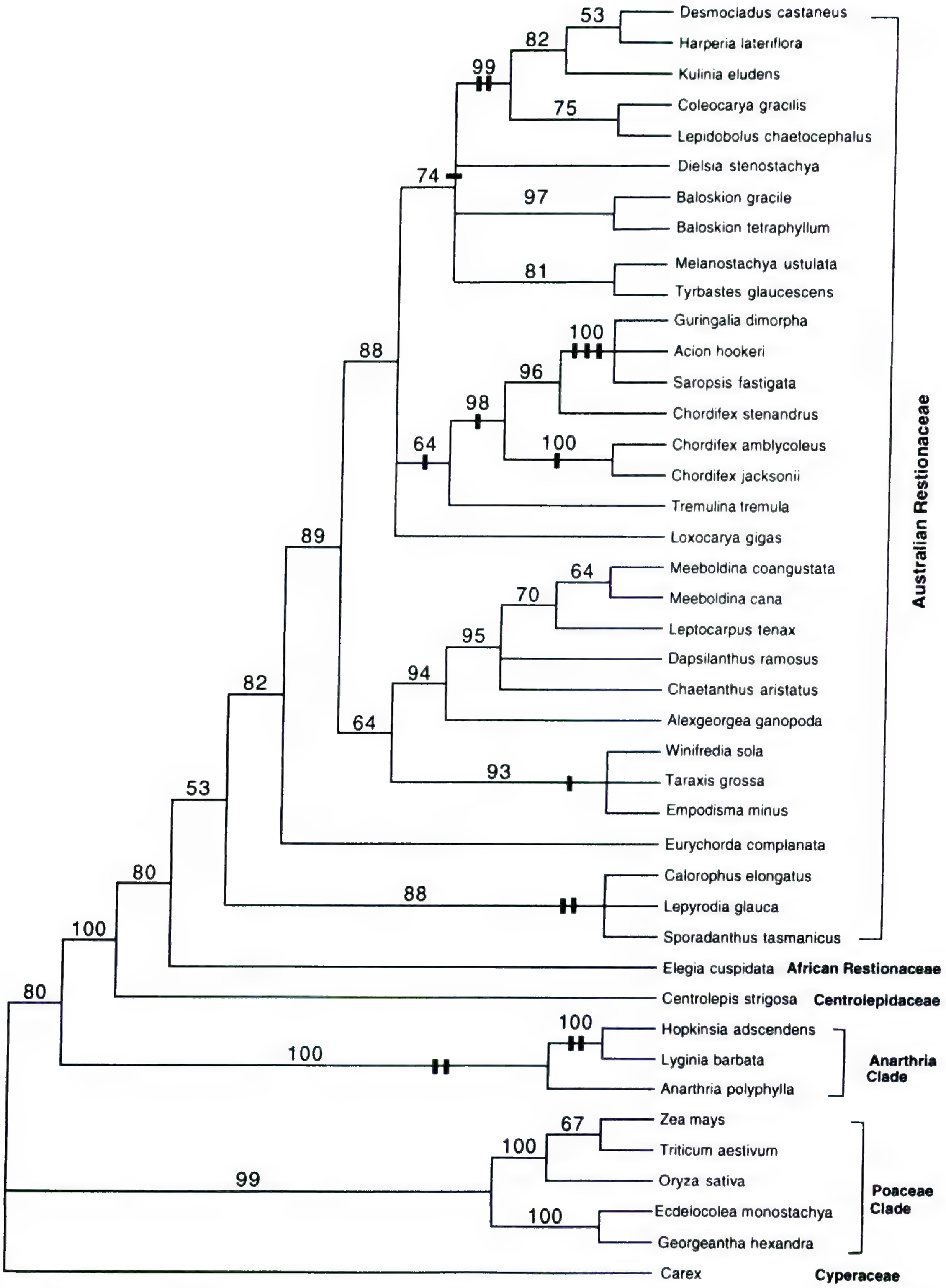

Figure 4. Jackknife consensus tree for Restionaceae and allied families from parsimony analysis of chloroplast DNA data. Numbers indicate jackknife support for individual nodes. Cross-bars indicate unique (non-homoplasious) indels, in the *trn*L intron or *trn*L-*trn*F spacer, that are synapomorphies for clades.

plant families. Neither of these examples is typical of the situation in the Australian flowering plants.

Wollemia created unprecedented public, media, and scientific interest. It raised public enthusiasm, awareness, and knowledge of environmental and biodiversity conservation issues. Its importance and rarity make it a wonderful example in education programs and political contexts. It has emphasized to the community the need for habitat conservation in species survival and given a focus for programs to explain the nature of biological research. To investigate its significance and conservation there has been research in systematics and evolutionary relationships, palaeontology, ecology, genetics, plant pathology, mycology, and plant propagation; this has helped to publicize the role of all these disciplines (Hill, 1996). School groups, government members, and the general community are enthusiastic about seeing the plants, so it has raised the profile of our botanic gardens and of their scientific and educational programs; it has also been a major profile-raiser for the New South Wales National Parks and Wildlife Service. *Wollemia* has contributed to understanding of structures in fossil Araucariaceae and conifer-mycorrhizal associations; its survival has added to the picture of long-term regional floristic change, perhaps even a step in the regional replacement of coniferous vegetation by flowering plants. After an extended period of small population size, it shows no discernible inter-plant genetic diversity.

Our other example, Restionaceae and allies, was probably the most neglected of all substantial Australian flowering plant groups and so was the richest site remaining for new discoveries. Distinguishing the many new rare species permits a focus to be developed on their conservation needs. Better knowledge of relationships within the Restionaceae clarifies an instance of the distinction between the intercontinental links that date from at least Gondwanic times and those that may represent relatively recent long-distance dispersal. In addition to greater understanding of Southern Hemisphere floras, there is improved understanding of relationships among the families of the Poales, with the recognition of the *Anarthria* clade and further demonstration (Fig. 4) of the closeness of Ecdeiocoleaceae to Poaceae, in agreement with Doyle et al. (1992).

Both examples are significant, but in each case the significance of the discoveries is only fully realized in the context of knowledge of organisms and their evolution that has been established by research in many fields. The history and antiquity of the Araucariaceae make *Wollemia* so notable. Similarly, relationship to the Poaceae adds to the relevance of findings in the Restionaceae. The historical biogeography of world floras, especially those of the Southern Hemisphere, provides a context for discoveries in both these groups; findings in these groups, in turn, clarify aspects of the development of these floras.

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FLORISTIC SURPRISES IN NORTH AMERICA NORTH OF MEXICO[1]

Barbara Ertter[2]

Abstract

Contrary to recurring perceptions that the flora of North America north of Mexico has been fully explored and cataloged, the rate of ongoing discoveries has remained remarkably constant for much of the last century and shows no evidence of tapering off. This is particularly evident in western and southeastern North America, where dramatic new species and occasional monotypic genera are still coming to light, even along highways and near major cities. Furthermore, the same level of ongoing discovery also characterizes other aspects of floristic information, including the distribution of rare species and the occurrence of invasive pest plants. The majority of ongoing discoveries are dependent on individuals and organizations operating outside of academia, with declining opportunities for formal training in floristics or access to scientific expertise when complex situations are encountered. This situation is connected to the perception of floristics as rote data compilation, when it is in fact better understood in the context of a massive attempt to model biodiversity, resulting in an intricate suite of nested hypotheses that are constantly being tested and modified. The incompleteness of our floristic knowledge takes on critical significance in an era when decisions are being made that will irrevocably determine the fate of our national floristic heritage. The cost of this ignorance can cut multiple ways, increasing the risk of misplaced mitigation efforts as well as avoidable loss of irreplaceable biodiversity. Although the magnitude of the task is daunting, significant advances are achievable in a collaborative framework, which would yield a vastly improved floristic knowledge base for informed decision-making.

Key words: biodiversity, floristics, North America, vascular plant flora.

Underlying much of our current land-use management planning, legislation, funding allocations, and hiring decisions is the assumption that the flora of North America north of Mexico (for brevity's sake, hereafter referred to simply as "North America") has been fully explored, cataloged, and mapped, at least to the extent that is needed for informed decision-making. Or, to the extent that exceptions are allowed, it is assumed that such knowledge accumulates in the form of static data sets, descriptive rather than truly scientific in nature, and further that the existing academic infrastructure is adequately addressing the gaps in our floristic knowledge. This paper sets out to challenge these assumptions, as well as the equally common perception that floristic surveys inevitably represent a threat to private landowners. The first half of the paper provides testimony to the wealth and diversity of ongoing floristic surprises in North America, whereas the second half addresses the factors that influence these discoveries and the resultant implications.

The majority of statistics and examples that form the basis of this paper are derived from the author's personal expertise and vascular plant focus. The resultant western North American bias should not, however, obscure the fact that this region is a rich source of ongoing novelties. An effort has nevertheless been made to include examples from other geographic areas and representing other groups traditionally studied by botanists: bryophytes, algae, fungi, and lichens. The proportional representation of examples should not be taken as an accurate reflection of actual discoveries among geographic areas and plant groups, or of their significance to science or land-management issues.

Assumption 1: The Fully Cataloged Flora

A. HISTORICAL PERCEPTIONS

The perception that the vascular plant flora of North America has been fully explored and cataloged has a surprisingly long history, as analyzed from our current state of knowledge. As early as 1858, Thomas Bridges, an Englishman collecting in California, wrote the following to Sir William J. Hooker (quoted in Jepson, 1933):

> "I can scarcely describe to you how pleasing and gratifying it has been to me to learn that in my collections you have found some new and rare plants—I was partially under the impression that from the labours of Douglas, Hartweg, Jeffrey, Lobb and other travelers

[1] Excerpts from the October 1998 presentation on which this paper is based have been subsequently highlighted in several media publications, notably *U.S. News and World Report* (Tangley, 1998) and *Science News* (Milius, 1999).

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> from Europe with the many United States Exploring Expeditions that little or nothing remained to be discovered and only gleanings were left to those of us of the present day."

As it happens, the "gleanings" left by Bridges's predecessors comprised the majority of California's flora as currently known. In fact, the number of known species increased by one-fourth during the subsequent two decades under the auspices of the California Geological Survey, primarily due to the efforts of William H. Brewer and Henry Nicholas Bolander. The two-part botanical report of the California Geological Survey (Brewer et al., 1876; Watson, 1880), which represented the first comprehensive flora of California, included full entries for approximately 3450 vascular plant taxa. This contrasts both with the initial estimate of 2000 (as noted in Whitney's introduction to the first volume) and the latest tally of 7036 vascular plant taxa recognized as occurring outside of cultivation in California (Hickman, 1993). Not only were there only half the number of taxa known in 1880 as in 1993, but there is by no means a strict one-to-one correspondence within the apparent overlap, primarily due to misapplied names and non-persisting introductions.

As it happens, Bolander was himself guilty of seriously underestimating what still lay waiting to be found, when he challenged Alphonso Wood's claim of collecting 1490 species of flowering plants on a journey from San Diego up the coast and through northern California in 1866. In an address to the California Academy of Natural Sciences, "Professor Bolander considered it probable that there were not over 500 species of flowering plants actually existing in that part of California" (Leviton & Aldrich, 1997: 87). On the contrary, well over 4000 taxa of vascular plants are now known to occur in the biogeographic subdivisions of California that Wood traversed (as calculated from Hickman [1993] by R. L. Moe, pers. comm. 1998), though how many of these Wood actually encountered is admittedly another matter.

Bolander's attitude was in full sway several decades later, when Katharine Brandegee accused Edward Lee Greene of conflating California's flora, with the statement, "It is safe to say that not more than one in ten of [Greene's] species is tenable, and probably one in fifteen or twenty would be nearer the mark" (Brandegee, 1893: 64). It turns out that Brandegee was actually the one who was way off the mark, in that a respectable 70% of Greene's taxa, at least those described while he was residing in California, have stood the test of time (McVaugh, 1983).

A marvelous anecdote relayed by Heller (1908: 12–13) from one of his correspondents shows just how well ensconced was the general belief that the North American flora had been fully cataloged by the end of the 19th century:

> "[M]y first botanical work was done in California, where my teacher was looked upon by me as the complete essence of knowledge, and everything she said was right—and such is often the case when one is fourteen years old . . . . At the time I used to range over territory [in the mountains near San Diego] which probably was not searched over botanically or ornithologically before nor since . . .. Sometimes as a result I would return with 30 or 40 plants, and after vain attempts to name them in my botany (Rattan's Popular Flora) would take them to the teacher. The usual words which took place were about as follows on the teacher's part: 'Can't you find these in the botany?' 'No.' Study of the specimens and consulting the botany followed on the teacher's part, with the usual ending by her saying: 'They are not given in the botany. They are not good for anything on that account. Throw them away.' "

As a final example in the botanical lore, one of Brandegee's supporters, Marcus E. Jones, is said to have commented that "he felt sorry for all future generations of botanists because he [Jones] had named all the western American taxa, and there would be nothing left for them to do" (S. Welsh, pers. comm. 1998). To the contrary, the rate of discovery of rare plants in Jones's home base of Utah remains high, with a significant peak in the 1980s (Stone, 1998; Fig. 1).

In essence, the inclination to believe that the era of floristic exploration in North America is over apparently has an inherent persistency to the point of becoming a psychological phenomenon worth investigating in its own right. In the words of Stan Welsh (pers. comm. 1998), "Each major publication on western plants has left the impression that all of the work has been done, that nothing remains to be discovered, that everything worth naming has been named." The perceptions of the 19th century have accordingly become the dogma of the 20th century, in which the common understanding is that the flora of North America has, with the rare exception, been fully explored, cataloged, and mapped (Reveal, 1991). At its worst, the attitude developed that anyone describing new species of plants from North America was indulging in species conflation for the sake of ego gratification, rather than practicing valid science.

Against this tide, there have admittedly been some voices to the contrary. In his introduction to the second volume of the botanical report of the

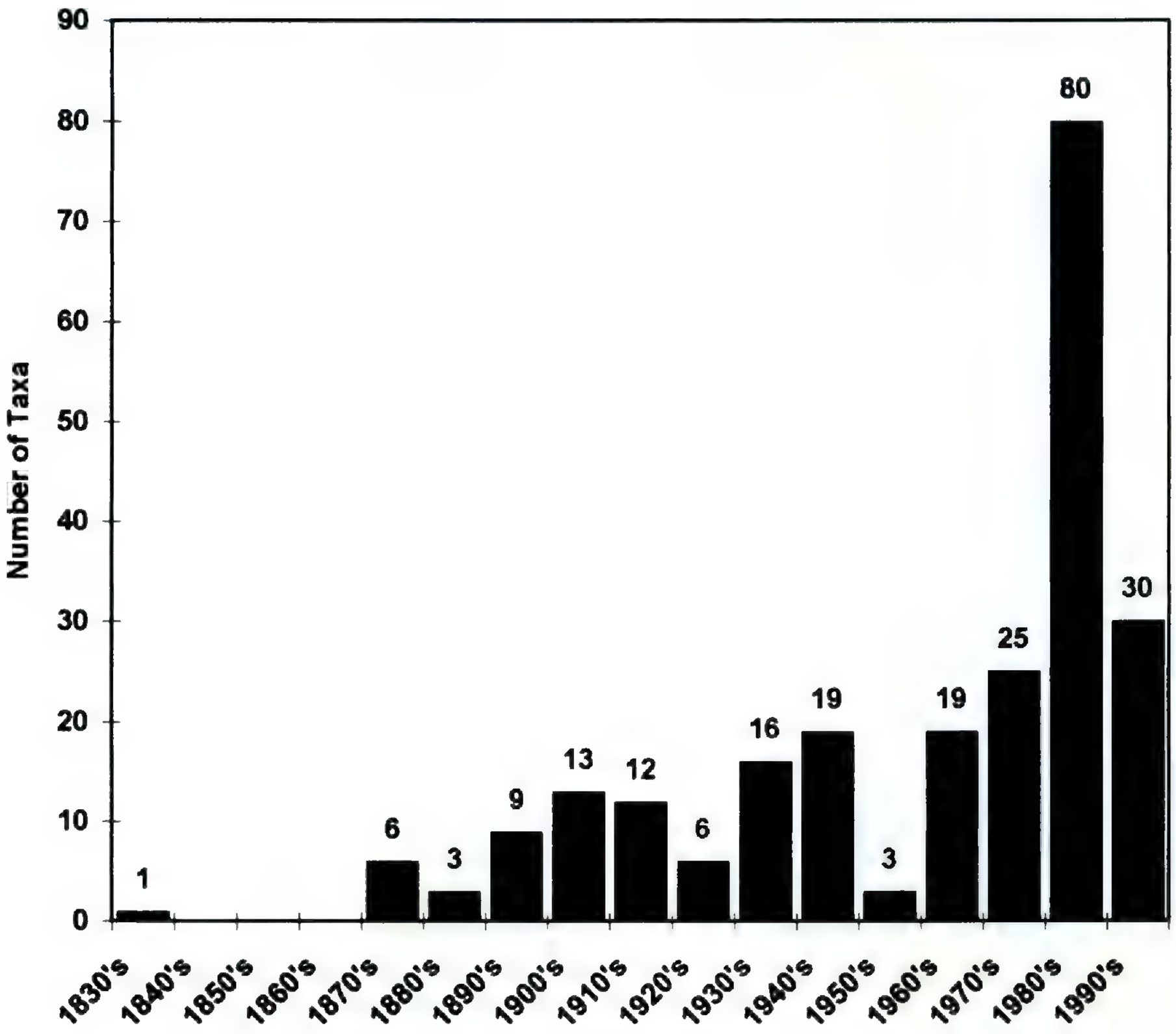

Figure 1. History of rare plant description in Utah (figure and caption prepared by R. D. Stone). Bars represent the number of rare vascular plant taxa (species, subspecies, and varieties) in Utah that were formally described within each 10-year period. Rare plants (N = 242) are defined as those taxa "with known or suspected range-wide viability concern" (Stone, 1998). The data show that most rare plants in Utah are recently described. This is understandable for two reasons: (a) plants are often considered rare when they are first described but tend to be removed from rare lists as they become better known; and (b) after more than a century of plant exploration and discovery in Utah, the plants now being described tend to be "the rarest of the rare."

Geological Survey of California, Watson (1880) indicated, "There still remains ample opportunity for good botanical work at almost any locality among the mountains, hills, and valleys of the State, to which it is hoped that these volumes may prove both an incentive and an aid." And, in a summation lecture of a symposium on the Broadening Basis of Classification, Lincoln Constance (1964) noted, "Many otherwise informed persons assume that the exploratory phase of botany is essentially complete; this assumption is, of course, entirely erroneous."

B. STATISTICAL CHALLENGES

(1) Shevock & Taylor (1987)

Possibly the first statistical challenge to the common perception was that of Shevock and Taylor in 1987, provocatively titled "Plant exploration in California: The frontier is still here." In it, the authors tallied 219 vascular plants described from California for the two decades from 1968 to 1986, an average of 11 taxa per year. Taylor (pers. comm. 1998) has continued the analysis, demonstrating that the rate of discovery remains constant (Fig. 2). He further extrapolates that, if the rate of discovery begins to taper off right now and follows the curve displayed by more fully cataloged parts of North America (e.g., the northeastern United States), a *minimum* of 300 or more undescribed vascular plant taxa are still waiting in the wings in California alone (Fig. 3).

(2) Hartman & Nelson (1998)

Furthermore, although California clearly leads the pack, a recent publication by Hartman and Nelson (1998) demonstrates the pervasiveness of ongoing floristic discovery throughout North America. For the two decades from 1975 through 1994, a total of 1197 vascular plant taxa were described as

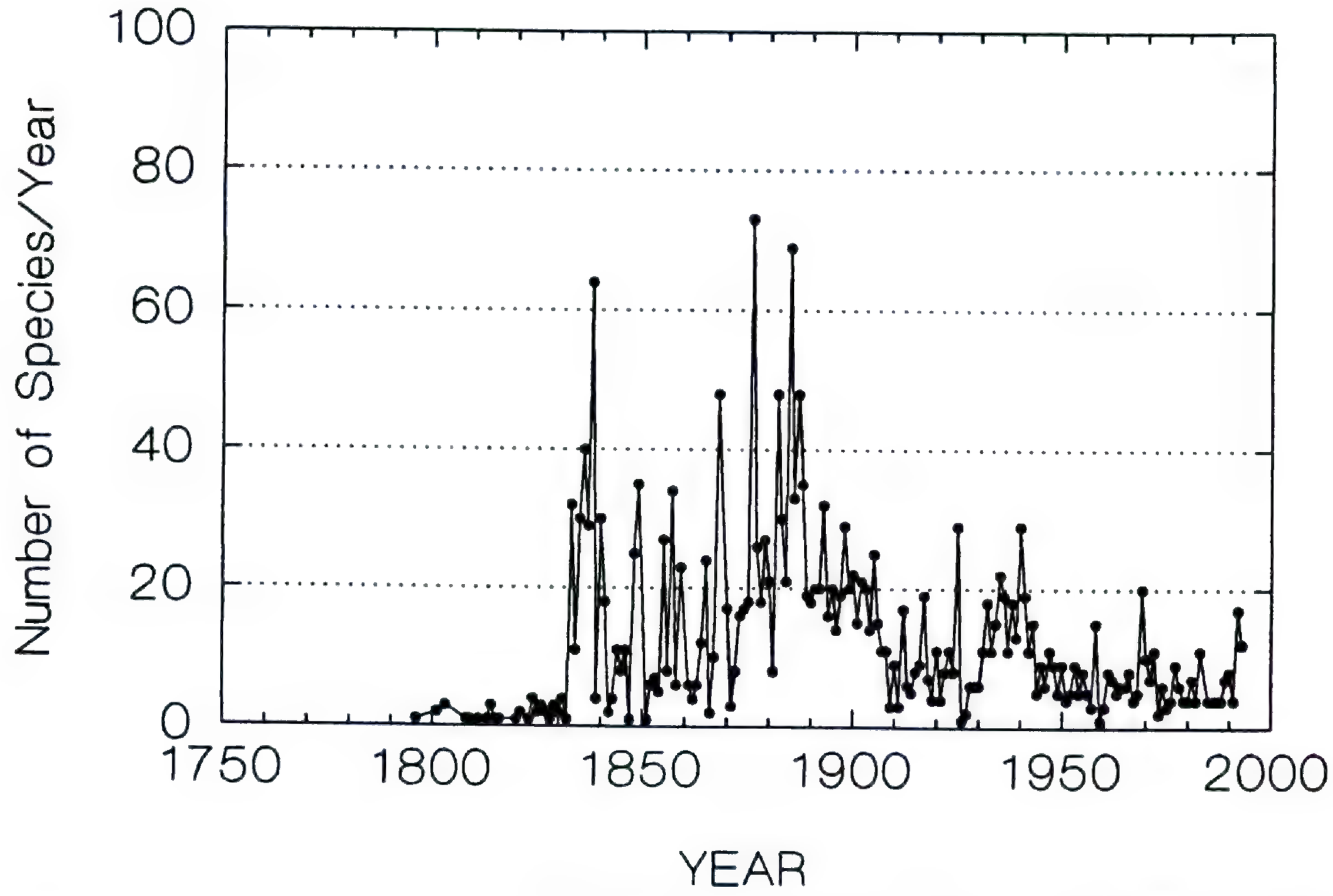

Figure 2. Yearly description rate of endemic species of plants in California and/or the California Floristic Province (prepared by D. W. Taylor, unpublished data).

new to science, ranging from monotypic genera to formae and nothotaxa (collectively referred to as "novelties"). The 603 full species comprise 3.21% of the 18,781 currently estimated to occur in North America (1998 estimate provided by *Flora of North America North of Mexico*). The overwhelming majority are from the western and southeastern United States, but essentially all states and provinces contributed to the total (including a forma from Rhode Island, *Lindera benzoin* f. *rubra* R. L. Champlin). Most are angiosperms, but 78 pteridophytes and 6 gymnosperms are represented.

Other statistics compiled by Hartman and Nelson included:

Number of holotypes by political unit (excluding formae and nothotaxa). Top 10 = California (217), Utah (183), Texas (70), Nevada (63), Arizona (57), Oregon (42), New Mexico (41), Florida (38), Idaho (33), and Wyoming (32).

Families with the greatest number of novelties (excluding formae and nothotaxa). Top 10 = Asteraceae (186), Brassicaceae (91), Fabaceae (84), Scrophulariaceae (46), Polygonaceae (46), Poaceae (44), Cactaceae (36), Liliaceae (30), Apiaceae (27), and Lamiaceae (26).

Authors of novelties. Top 10 = S. L. Welsh (118), R. C. Rollins (62), J. L. Reveal (45), R. C. Barneby (32), G. L. Nesom (26), N. H. Holmgren (25), W. H. Wagner (24), B. L. Turner (23), S. Goodrich (19), and B. Ertter (18).

Taking into consideration that not all published novelties are subsequently accepted as worthy of taxonomic recognition, Hartman and Nelson accordingly calculated the acceptance rate in a variety of taxonomic works, ranging from 63% to 98%, with somewhere around 90% apparently being the norm. This may in fact be an underestimation, if a recent study by Windham and Beilstein (1998a, b) is any indication, ironically involving the two leading authors of novelties. Lest anyone assume that Welsh's impressive total (nearly double that of Rollins's) results from a bad case of species conflation, Windham and Beilstein give strong evidence that Welsh erred on the conservative side in at least one instance. Not only did the elegant convergence of micromorphological, molecular, and other evidence show that Welsh mistakenly lumped some of Rollins's species of *Draba* (Brassicaceae), but furthermore indicated that Rollins himself had confused taxa that were morphologically convergent but only distantly related.

Parallel to Taylor's analysis, Hartman and Nelson showed that the rate of publication of taxonomic

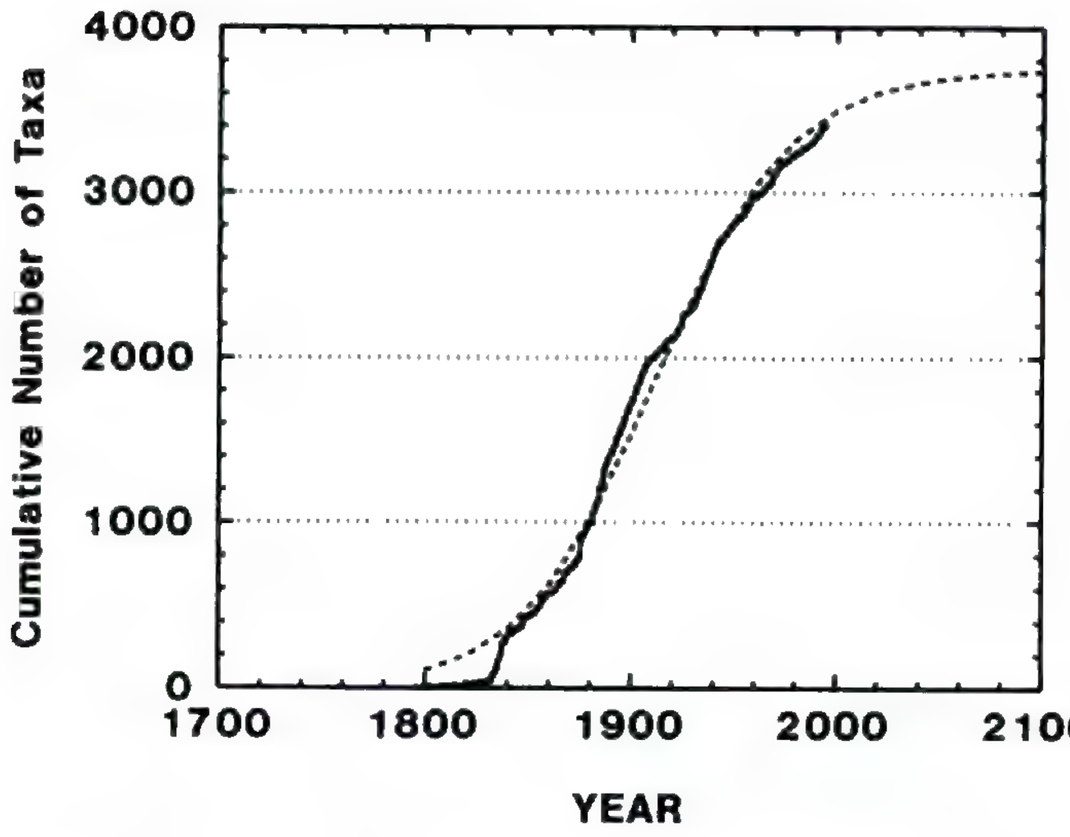

Figure 3. Curve depicting the historical trend in discovery of endemic plants of the California region and an approximative extrapolation of a possible future trend (fig. and caption prepared by D. W. Taylor). The solid line shows the rate of accumulation of published names through publication of *The Jepson Manual* (Hickman, 1993). The data set includes those endemic taxa as recognized in Hickman (1993) or Skinner and Pavlick (1994), plus some 18 taxa found in the Oregon portion of the California Floristic Province, 120 taxa found in the Baja California portion, and 2 taxa found in the Sierra Nevada portion of Washoe County, Nevada. The fitted curve (dashed line) was obtained by iterative nonlinear estimation modeling (employing Wilkinson, 1990) with a cumulative logistic assumption of curve shape. The assumed diminishing-return curve shape was selected based on the observed trends in decline of novelties for the vascular flora of New England and North American conifers (Taylor, unpublished data). The best-fit solution predicts a leveling-off in about 90 years, after an additional 298 taxa have been described. The model shown here is conservative, forcing a decline to equilibrium coincident to publication of *The Jepson Manual* in 1993. The observed trend shows no direct evidence of such a decline, however, in that an additional 26 new taxa have been described between 1994 and 1998 (D. Taylor, unpublished data).

novelties has remained relatively constant since 1955, averaging nearly 60 per year. They predicted, however, that the rate of publication of novelties will diminish once *Flora of North America North of Mexico* (FNA) is completed. It is accordingly worth noting that Taylor's statistics do not show a comparable drop following the appearance of *The Jepson Manual* (Hickman, 1993); if anything, the appearance of an up-to-date flora has spurred essential fieldwork and increased the likelihood of recognizing a novelty as such. There is at least one example already that the appearance of a generic treatment in FNA is independent of ongoing discoveries: Warren H. and Florence Wagner (1994; pers. comm. 1998) report that they have already accumulated six new species of *Botrychium* beyond the 30 included in their treatment for FNA (Wagner & Wagner, 1993).

Although it is impossible to know exactly how much remains unknown, a calculation based on Taylor's method of extrapolation has at least some conceptual validity. If Taylor's extrapolation of 300+ still-to-be-discovered vascular plant novelties in California is accurate, and if the 1:6 ratio of California-to-North America novelties remains constant, then at least 1800 more novelties can be expected for North America. If half are full-fledged species (as in Hartman and Nelson's analysis), then nearly 5% of the North American vascular plant flora is still undescribed!

(3) Non-vascular plants and fungi

Comparable statistics have not previously been published for non-vascular plants and fungi, but Marshall Crosby and Raymond Stotler have compiled the raw numbers on mosses and liverworts, respectively, kindly making them available for this paper. From 1975 to 1998, a total of 63 mosses were described from North America, including 42 full species (M. Crosby, pers. comm. 1998). This represents 3.17% of the 1323 species estimated for FNA, almost the same percentage as for vascular plants (3.21%). Liverworts tell a similar story, with 19 novelties described in the last 20 years, representing 3.44% of the current tally of 553 (R. Stotler, pers. comm. 1998).

Statistics generated from the *Index Nominum Algarum*, maintained by Paul Silva and Richard Moe (pers. comm. 1999), indicate that at least 63 marine macroalgae have been described from the North American coastline since 1980, with an incomplete cataloging of pre-1986 publications. This clearly indicates that algae are also still being actively discovered and identified, perhaps at even higher percentages than for vascular plants. Comparable summaries have not been generated for lichens or fungi, which in general lag behind vascular plants in terms of systematic research.

[*Note.* Although it is now well established that "plants" are represented by at least three distinct lineages (green, red, and brown), and that fungi are more closely related to animals than to green plants (e.g., the topic of a keynote symposium on "Phylogeny of Life" at the XVI International Botanical Congress), there is no evidence that these groups are being evicted from herbaria or the research realm of botanists. Their inclusion in this paper is furthermore justified by the burgeoning move to treat bryophytes and lichens, and potentially fungi and algae, under the same conservation umbrella as currently exists for vascular plants, with the issues addressed by this paper of relevance to all.]

C. EXAMPLES OF "NEW TO SCIENCE" DISCOVERIES

Although the statistics cited above are impressive in their own right, it is not evident to what extent strikingly new discoveries are represented, as compared to cryptically distinct variants of marginal significance. To address this question, a selection of the most dramatic of the newly discovered and/or described taxa are presented here, drawn from among the 1197 novelties tallied by Hartman and Nelson and numerous others published since 1994. Choosing among the wealth of riches was one of the more challenging parts of preparing this paper, with new examples constantly coming to the fore. The resultant choices are organized among the following categories:

(1) Monotypic genera

Five species covered by Hartman and Nelson were distinctive enough to be described as new monotypic genera: *Apacheria chiricahuensis* C. T. Mason (Crossosomataceae), *Cochisea robbinsorum* W. H. Earle (Cactaceae) (subsequently included within *Coryphantha* by Benson [1982]), *Dedeckera eurekensis* Reveal & J. T. Howell (Polygonaceae), *Shoshonea pulvinata* Evert & Constance (Apiaceae), and *Yermo xanthocephalus* Dorn (Asteraceae). *Apacheria* and *Cochisea* are from Arizona, *Shoshonea* and *Yermo* are from Wyoming, and *Dedeckera* is from California. *Apacheria,* found in the Chiricahua National Monument in 1973, became the second genus assigned to the family Crossosomataceae (Mason, 1975). *Yermo,* whose closest probable relatives grow in deciduous forests in eastern North America, is known from a single remote population located along a proposed pipeline route in the Wyoming desert (Dorn, 1991). *Shoshonea,* discovered by retired schoolteacher and rock-gardener Erwin Evert in 1979, has more recently been found to occur within two miles of downtown Cody, Wyoming (Evert & Constance, 1982; R. Hartman, pers. comm. 1998). The distinctive summer-blooming shrub *Dedeckera* was discovered by conservation activist Mary DeDecker (Reveal & Howell, 1976; Nilsson, 1994), with one population now known within walking distance of a scientific research station on the outskirts of Bishop, California.

Subsequent to Hartman and Nelson's publication, two more monotypic genera of vascular plants have been described, by coincidence both Brassicaceae from California. The first was *Sibaropsis hammittii* S. Boyd & T. S. Ross, with three separate occurrences encountered in the course of doing an environmental impact survey in relatively well-botanized and well-traveled portions of southern California (Boyd & Ross, 1997). The second was also discovered by a consultant in an area proposed for development, within earshot of Interstate Highway 5 in the San Joaquin Valley. Originally suspected of being yet one more introduced European annual, it was determined instead to represent a unique new species and was accordingly described as *Twisselmannia californica* Al-Shehbaz (Al-Shehbaz, 1999).

Looking beyond vascular plants, newly discovered species distinctive enough to be described as new genera are particularly common among algae. Of the 63 macroalgae cited above, eight were distinctive enough to be described as new genera: *Binghamiopsis caespitosa* I. K. Lee, J. A. West & Hommers from California; *Boreothamnion villosum* M. J. Wynne and *Orculifilum denticulatum* S. C. Lindstr. from Alaska; *Calliclavula trifurcata* C. W. Schneid. and *Nwynea grandispora* Searles from North Carolina; *Chlorojackia pachyclados* R. Nielsen & J. A. Correa from Nova Scotia; *Rhododraparnaldia oregonica* Sheath, Whittick & K. M. Cole from Oregon; and *Verosphacela ebrachia* E. C. Henry from Florida.

Among bryophytes, *Ozobryum ogalalense* G. L. S. Merrill (Pottiaceae) was also recently discovered and described as a monotypic genus (Merrill, 1992). Although it was subsequently transferred to *Molendoa* (Zander, 1993), its distinctiveness as a species remains noteworthy. The new moss is furthermore remarkable in making its appearance in the Great Plains, a region otherwise relatively depauperate in both mosses and new discoveries in general.

(2) Charismatic megaflora

The award for most publicity for a recent discovery goes to the Shasta snow-wreath, *Neviusia cliftonii* Shevock, Ertter & D. W. Taylor (Rosaceae), whose serendipitous discovery in 1992 in northern California made both *The New York Times* (2 February 1993) and the *Frankfurter Allgemeine* (30 December 1992) based on two aspects of particular significance (Shevock et al., 1992). First, the Shasta snow-wreath was the second species in a genus that had previously been known as a single rare species in the southern Appalachians, over a thousand miles away. This disjunct distribution, indicative of a Tertiary relict, was, however, less puzzling than the second aspect: namely, that this never-before-collected shrub was locally co-dominant (with poison-oak) along a well-traveled highway, 25 miles northeast of Redding, California. Once a focused

search was undertaken, several additional populations were readily located, one adjacent to a developed campground. As a further anomaly, the currently known range lies largely within one of the few areas (Redding quadrangle) whose vegetation types were completely mapped and published (Weislander et al., 1939); nevertheless, *Neviusia* was not among the abundant vouchers.

As an eastern example of "charismatic megaflora," a 5–7-m-tall rosaceous shrub or small tree, discovered in Arkansas in 1970 and initially identified as either a *Crataegus* or an *Aronia*, was subsequently described as *Mespilus canescens* Phipps (Phipps, 1990). As such, it was a surprising addition to a previously monotypic genus known only from Europe, the medlar *Mespilus germanica* L. Subsequent isozyme studies supported the inclusion of the new species within *Mespilus* (Phipps et al., 1991). Described as being "of exceptional beauty," only 25 individuals are known from a single 22-acre grove.

Another contender for most charismatic recent discovery from eastern North America is the Kentucky lady's slipper, *Cypripedium kentuckiense* C. F. Reed (Orchidaceae), among the tallest and showiest species in a genus of showy orchids. Although currently known from several southeastern states, it was not described until 1981, long after the author had first encountered it cultivated in a garden (Reed, 1981). The most recently discovered population, in coastal Virginia in 1995, is only 150 km as the crow flies from the center of Washington, D.C. (Weldy et al., 1996).

(3) Botanical "hot spots"

A jackpot of undescribed species is occasionally encountered in areas of complex geology and rugged terrain, which set the stage for an often bewildering expression of island biogeography in a continental setting. In such areas, an unusual substrate or isolated mountaintop has the potential of harboring a unique suite of endemic plants, and the first botanist to reach the site can reap a bonanza of floristic surprises.

As it happens, my own career as a professional taxonomist began when I had the good fortune of participating in the discovery of one such botanical treasure trove while still an undergraduate, thanks to a newly built gravel road in the early 1970s that made Leslie Gulch in southeastern Oregon a two-hour drive by passenger car from Boise, Idaho. The unique ash-flow tuffs of Leslie Gulch have thus far yielded a total of five plant taxa new to science: *Senecio ertterae* T. M. Barkley, *Mentzelia packardiae* Glad, *Ivesia rhypara* Ertter & Reveal, *Artemisia packardiae* J. W. Grimes & Ertter, and *Phacelia lutea* var. *mackenziorum* J. W. Grimes & P. L. Packard (Grimes, 1984). Some of these species are so distinctive that their relationships remain unclear, whereas variants of several other species might prove to be taxonomically distinct upon further study. In addition, Leslie Gulch turns out to be the "mother lode" for several other species that had been known previously from only a handful of populations: *Trifolium owyheense* Gilkey, *Astragalus sterilis* Barneby, and *Eriogonum novonudum* M. Peck.

Alas, Leslie Gulch is now overshadowed by the latest "hot spot," the Ketona Glades in Bibb County, Alabama. In 1992, a group of environmental consultants undertaking a rare plant survey by canoe on the Little Cataba River came upon a calcareous glade community harboring at least seven undescribed taxa: *Spigelia gentianoides* Chapman ex A. DC. var. *alabamensis* K. Gould, *Onosmodium* sp. nov., *Erigeron strigosus* var. nov., *Dalea* sp. nov., *Castilleja* sp. nov., *Liatris* sp. nov., and *Silphium* sp. nov. In addition, several state records for Alabama were present, most with some level of formal rarity status, along with a new county record for the federally endangered *Xyris tennesseensis* Kral (Allison, 1994; Gould, 1996).

The rugged southern Sierra Nevada in California also continues to be a rich source of novelties, with three new taxa discovered in a single day on a remote marble ridge in 1996: *Heterotheca monarchensis* D. York, Semple & Shevock, *Gilia yorkii* Shevock & A. G. Day, and a still-undescribed *Eriogonum*. The ridge also harbors the only known California populations of three mosses (J. R. Shevock, pers. comm. 1998).

(4) In our backyards

Although the majority of new discoveries are encountered in relatively remote sites, a surprising number appear around significant population centers, even those with major herbaria and a long tradition of botanical exploration. Among the more dramatic of these "in our backyards" examples are the following:

- Morefield's leather-flower, *Clematis morefieldii* Kral (Ranunculaceae), was discovered in 1982 by a budding botanist who practiced by collecting specimens around his neighborhood inside the city limits of Huntsville, Alabama (Kral, 1987). Now federally listed as Endangered, the species is still only known in and near residential areas in the Huntsville area. Morefield himself, inspired by this

early experience, went on to become botanist for the Nevada Natural Heritage Program.

- Not far from Las Vegas, Nevada, in the course of a floristic survey of Red Rock Canyon National Conservation Area, *Ionactis caelestis* P. J. Leary & G. L. Nesom (Asteraceae) was discovered in 1990. The highly localized population, within sight of the Las Vegas casinos, occurs on an edaphic island of sandstone surrounded by limestone. The species is distinctive enough that it might justifiably be treated as a monotypic genus (Nesom & Leary, 1992).
- Approximately 25 miles west of downtown Los Angeles, California, a few miles north of the trendy community of Malibu in the Santa Monica Mountains, *Baccharis malibuensis* R. M. Beauch. & Henr. (Asteraceae) was discovered in 1988 (Beauchamp & Henrickson, 1996). Beauchamp had earlier described another *Baccharis, B. vanessae* R. M. Beauch., from the midst of housing developments in central San Diego County (Beauchamp, 1980).
- Only about six miles from one of the main herbaria in Utah, on cliffs behind some summer cabins, *Viola franksmithii* N. H. Holmgren (Violaceae) was discovered in 1989 by a "modern-day naturalist" who was monitoring other rare plants known to occur in Logan Canyon (Holmgren, 1992). Disagreement exists as to taxonomic placement of this distinctive species (H. J. Ballard, pers. comm. 1998).
- A short distance outside of Yosemite National Park, among the most-visited parks in the United States, the showy-flowered *Erythronium taylori* Shevock & G. A. Allen (Liliaceae) was discovered in 1996, bringing to three the number of extremely local endemic *Erythronium* in the lower Sierra Nevada (Shevock & Allen, 1998). In addition to being the only New World species of the genus with scented flowers, *E. taylori* is intriguing in being a candidate for the *Erythronium* once reported to occur in Yosemite Valley (Brandegee, 1891), where no representatives of the genus are currently known (D. W. Taylor, pers. comm. 1998).
- In spite of growing less than 10 miles from downtown San Francisco, California, in an extensively botanized county with a recently updated flora (Howell, 1970), *Calochortus tiburonensis* A. J. Hill (Liliaceae) was not discovered until 1972. The species is so distinctive that "its existence challenges the currently accepted infrageneric classification" (Hill, 1973: 104). The protologue furthermore noted, "That a previously uncollected new species . . . was discovered in such a botanically well known area suggests the need for a very careful look at any areas that are threatened by development or other disturbance, especially near expanding population centers. Had this species not been noticed soon, it might very well have become extinct without ever having been recorded."
- Nestled among the Lick Observatory complex on Mount Hamilton, within easy reach of the University of California at Berkeley, the California Academy of Sciences, and Stanford University, *Lomatium observatorium* Constance & Ertter (Apiaceae) was not described until 1996, too late to be included in Hartman and Nelson's synopsis. Its location on Mount Hamilton was brought to the attention of Lincoln Constance, the expert on *Lomatium*, by a wildflower photographer, Nigel Hancock (Constance & Ertter, 1996).

(5) Species-rich genera

Lomatium, with 10 novelties listed in Hartman and Nelson, is also an example of a large genus that has undergone extensive speciation, resulting in an abundance of highly localized endemic species that are still being discovered at a steady rate. Among other significant examples are the following:

- *Astragalus* (Fabaceae) contains the largest number of novelties (43) listed in Hartman and Nelson, due in large part to the efforts of Rupert Barneby. *Astragalus* is also worth highlighting for the potential medicinal value of the new discoveries, given the known value of at least one Old World species, *A. membranaceus* Bunge. This species not only has a long history of use in Chinese traditional medicine, but it has also entered the American alternative medicine pharmacopoeia as an immune system enhancer, with some clinical evidence of activity in cancer patients with impaired immune responses (e.g., Chang et al., 1983; Kosuge et al., 1985). Intriguingly, this eerily approaches a case of fact following fiction, in that the hypothetical discovery of a cure for cancer in the form of *Astragalus*, only in this case a North American species, plays a key role in Duane Isely's fictionalized prognostication of what would happen if all taxonomists and their works suddenly disappeared (Isely, 1972).
- *Penstemon* (Scrophulariaceae): Where *Astragalus* has potential medicinal value, *Penstemon* has proven horticultural significance. The 29 novelties listed in Hartman and Nelson accordingly represent valued additions to the existing penstemon palette for rock-gardening enthusiasts, at least to the extent that they can be brought into cultivation without negatively impacting natural populations. In the Intermountain Region alone, seven species have been discovered and described subsequent to the 1984 treatment of *Penstemon* in the *Intermountain Flora*,

averaging one every two years (Holmgren, 1984, 1998).

- *Lesquerella* (Brassicaceae): Of the 83 species of *Lesquerella* in the late Reed Rollins's monumental synopsis of the Cruciferae of North America (Rollins, 1993), nearly half were authored or coauthored by Rollins himself over his long career, several in the book itself. As massive a compendium as this was, three additional species were discovered almost before the ink was dry: *L. lesicii* Rollins and *L. pulchella* Rollins from Montana (Rollins, 1995), and *L. tuplashensis* Rollins, K. A. Beck & Caplow from the Hanford Nuclear Reservation in Washington (Rollins et al., 1995). A short two years later, a fourth species, *L. vicina* J. L. Anderson, Reveal & Rollins, was published, with the epithet chosen in reference to the fact that the type locality was behind the home of a neighbor of one of the coauthors in Montrose, Colorado, growing in the sheep pasture (Anderson et al., 1997; J. Reveal, pers. comm. 1998).
- *Arabis* (Brassicaceae): The appearance of Rollins's (1993) compendium also triggered the description of a suite of four new *Arabis* from Canada, Alaska, and Greenland, as well as reports of numerous range extensions from the same region, all in the same publication (Mulligan, 1995). This example is significant in demonstrating that the northern latitudes are also full of floristic surprises, in spite of their generally fewer numbers of species. Farther south, *Arabis hirschbergiae* S. Boyd has recently been described from southern California, a stone's throw from a major highway (Boyd, 1998). All of these species are additions to the 17 novelties listed by Hartman and Nelson.
- *Eriogonum* (Polygonaceae): With 38 entries in Hartman and Nelson, *Eriogonum* has also proven to be an ongoing source of novelties, with one new species discovered on the Hanford Nuclear Reservation across the river from the new *Lesquerella,* as part of the same botanical survey (Reveal et al., 1995). An even more recent and dramatic example is provided by a pair of Friends of the Jepson Herbarium weekend workshops on *Eriogonum* in California in the summer of 1997, taught by *Eriogonum* specialist James L. Reveal. Of the 35 participants, mostly agency botanists and consultants, 3 ended up providing Reveal with additional undescribed taxa, leading to the quip that we should schedule a workshop on how to describe new species! In addition, Reveal confirmed that the variant of *E. nudum* Benth. that is the host plant for a federally listed butterfly, the Lange's Metal Mark *(Apodemia mormo langei* J. A. Comstock) is itself an undescribed taxon, bringing up to three the number of plants endemic to Antioch Dunes (a badly degraded inland dune complex on the edge of the Sacramento River delta in central California, less than an hour's drive from Berkeley; the other two are *Oenothera deltoides* Torrey & Fremont subsp. *howellii* (Munz) W. M. Klein and *Erysimum capitatum* (Douglas) Greene var. *angustatum* (Greene) Rossbach, both federally endangered.)
- *Carex* (Cyperaceae): *Carex* is noteworthy in that the majority of the 21 novelties listed in Hartman and Nelson, an average of 2 per year, are found in eastern North America. *Carex lutea* LeBlond, for example, was discovered in 1991 in North Carolina, where it is a rare endemic of wet savanna underlain by limestone. It is furthermore phytogeographically interesting in being a southern outlier (by 750 km) of a circumboreal species complex, possibly a relict from the Pleistocene. Associates of *C. lutea* include numerous other rare species, including Venus flytrap and an undescribed *Allium* (LeBlond et al., 1994).

Another recently described sedge, *C. juniperorum* Catling, Reznicek & Crins, is known from widely disjunct populations in Ontario, Ohio, and Kentucky. Although locally a groundlayer dominant, it was presumably overlooked because the inflorescences are nestled at the base of the plant and appear unexpectedly early in the season. In the protologue, the authors noted, "The recent discovery of this distinctive new species in a supposedly botanically well-known area suggests that even the flora of northeastern North America is not as well-known as is commonly supposed" (Catling et al., 1993).

(6) Scientifically significant discoveries

On top of the importance of cataloging the components of biodiversity for their own sake, many of the recently described species have carried significance beyond their intrinsic value. Some, such as *Neviusia cliftonii* and *Carex lutea,* pose interesting biogeographic puzzles. Others, including *Dedeckera eurekensis* and *Calochortus tiburonensis,* provide the key to unraveling phylogenetic questions (Reveal & Howell, 1976; Reveal, 1989a; Hill, 1973). *Dedeckera* is also significant as an ancient lineage postulated to have accumulated such a high segregational genetic load of heterozygosity that seed set is severely depressed (Wiens et al., 1989). Some additional examples:

- At the time of Keck's revision of *Ivesia* (Rosaceae) in 1938, *I. shockleyi* S. Watson was thought to be restricted to the Sierra Nevada of California and Nevada. Fieldwork by numerous botanists over

the last two decades, however, has shown not only that *I. shockleyi* is scattered on mountaintops across the Great Basin, with a varietally distinct outlier in Utah (var. *ostleri* Ertter), but that a previously unknown complex apparently represents the low-elevation analog on unusual edaphic sites (Ertter, 1989). The low-elevation complex consists of several closely related entities with widely disjunct distributions: *I. rhypara* Ertter & Reveal var. *rhypara, I. rhypara* var. *shellyi* Ertter, and *I. paniculata* T. W. Nelson & J. P. Nelson. This example of island biogeography in a continental setting is interpreted in the context of Pleistocene-driven isolation and radiation, with one lineage retreating to isolated montane "islands" and the other finding a comparable niche in unusual edaphic sites at lower elevations. Molecular investigations with Christopher Baysdorfer (California State University at Hayward) are currently under way to further elucidate the resultant evolutionary pattern.

- *Verrucaria tavaresiae* R. L. Moe is noteworthy not only in being one of the few known marine lichens, but also the only lichen known with a brown algal phycobiont. Described in 1997, it is another example of "in our backyards," occurring in the intertidal zone around San Francisco, California (Moe, 1997).
- *Calycadenia hooveri* G. D. Carr (Asteraceae), described in 1975, possesses a chromosome arrangement almost identical to that of *C. villosa* DC., which is unlike that of other species in the genus. Reconstructions of chromosomal evolution based on molecular phylogenies of *Calycadenia* show that the ancestor of both aneuploid species lineages (which comprise the bulk of the genus) had a chromosome arrangement similar or identical to that of *C. hooveri* or *C. villosa.* Without *C. hooveri,* the reconstruction of chromosome evolution would have been equivocal (Carr, 1975; Baldwin, 1993).
- Probably the most surprising discovery involving North American ferns has been the realization that several species of Hymenophyllaceae and Vittariaceae in the eastern United States exist primarily as gemmiferous gametophytes, either growing north of the range of the sporophytes or, in a couple of cases, with sporophytes produced rarely if ever (Farrar, 1993a, b). Although vegetatively reproducing gametophytes have been known since 1888, their relative abundance (10% of all fern species worldwide) and significance has only become appreciated relatively recently (e.g., Farrar, 1974). Once gametophytes became the target of attention, three new species were discovered in the early 1990s: *Hymenophyllum tayloriae* Farrar & Raine, *Trichomanes intricatum* Farrar, and *Vittaria appalachiana* Farrar & Mickel.
- The liverwort genus *Pellia* (Metzgeriales), perhaps second only to *Marchantia* in the amount of attention previously given to liverwort genera, nevertheless provides a case where a critical look at the "common" species in the field yields unexpected results. Prior to 1981, only four species were recognized worldwide; in relatively quick succession, however, two new ones were published from eastern North America (*P. megaspora* R. M. Schust. and *P. appalachiana* R. M. Schust. [Schuster, 1981, 1991]), and one more is currently being described from Mississippi (R. Stotler, pers. comm. 1998).
- The bryophyte *Takakia* has been a puzzle since its discovery in 1951, at which time it was considered to be a liverwort. However, only vegetative and archegonial material was known, and attempts to induce fertile structures in cultivation met with failure. Sporophytic plants of *T. ceratophylla* (Mitt.) Grolle were finally encountered in 1990, in the course of fieldwork in the Aleutian Islands, firmly establishing *Takakia*'s identity as a moss (Smith & Davison, 1993). In essence, floristic discovery resulted in the transfer of a genus from one division (Hepatophyta) to another (Bryophyta)!

D. OTHER KINDS OF FLORISTIC SURPRISES

Although newly described novelties capture the imagination, they represent only the tip of the iceberg of floristic surprises, only the starting point for the comprehensive information that is truly needed for making difficult decisions in a scientifically informed manner. Even more incomplete than our knowledge of what species exist is our knowledge of where they occur, what their habitat requirements are, and similar questions that can only be answered by extensive fieldwork coupled with critical taxonomic analysis.

An excellent example of the incomplete and nonstatic nature of floristic information is provided by the recently revised flora of Missouri, in which the number of plants known to occur in a relatively well-studied state (with one of the oldest and largest herbaria in the country) has increased by nearly 12% since 1963, two-fifths of them native (Yatskievych, 1999). Current research on the flora of Mount Diablo, an isolated mountain and popular state park situated 25 miles east of San Francisco, California, shows an even more dramatic increase. In spite of the high quality of the original floristic effort (Bowerman, 1944), a recent update (Bowerman & Ertter, in press) has increased the known taxa by 25%, approximately half of which are na-

tive. Furthermore, several species in the 1944 treatment have been deleted or replaced, as a result of misidentified vouchers or changed circumscriptions. On the other hand, locally occurring variants of *Eriogonum*, *Lomatium*, *Calystegia*, and *Gilia* have the potential of being undescribed novelties (Ertter & Schultheis, 1998).

Some of the main categories of "floristic surprises" other than novelties are the following:

(1) "Presumed extinct"

Nearly as dramatic as the discovery of new species is the rediscovery of species that had been thought to be extinct. A recent example in California is that of the Ventura Marsh milk-vetch, *Astragalus pycnostachyus* A. Gray var. *lanosissimus* (Rydb.) Munz (Fabaceae), which was recently found in Ventura County, California, after being presumed extinct for 40 years. Local newspaper coverage referred to "A botanical resurrection" *(Ventura County Star,* 21 Aug. 1997) and "The Elvis Presley of flowering plants" *(Santa Barbara News-Press,* 15 Aug. 1997). The wildlife biologist who found the plant, Kate Symonds, was quoted in one account as noting, "It is more common to realize something is gone that used to be around, rather than finding something thought to be gone that is still in existence. It feels like a second chance for the species" *(Sacramento Bee,* 15 Aug. 1997). Ironically, the site was a former oil field waste dump, dispelling any notion that significant discoveries occur only in pristine habitats.

Coincidentally, another recently rediscovered Californian *Astragalus*, *A. agnicidus* Barneby, was also associated with disturbance. In this case, a plant that had been deliberately eliminated because of its perceived toxicity to livestock *(agnicidus* = "lamb-killing") reappeared when logging activity apparently triggered the germination of seeds that had lain dormant for decades (Hiss & Pickart, 1992). This example also serves to illustrate the difficulty of determining presence versus absence of a species at a site, let alone globally, even when no mature individuals are evident.

The systematic search for selected subsets of the 416 plants and animals that are considered potentially extinct in the United States was given a major boost recently by the Canon Exploration Grants Program directed by The Nature Conservancy (Stolzenburg, 1998; Anonymous, 1998). Although a depressing majority have not been relocated to date, there have been enough satisfying success stories to justify the program, in more ways than one. As evidence, consider the following story transmitted by the director of the program, Bruce Stein (pers. comm. 1998):

> "As I was jotting my note to you, a second 1998 find from the Canon program was slipped into my box. This just in from South Texas near Corpus Christi: *Paronychia lundellorum* B. L. Turner [Caryophyllaceae], which was last collected in 1958. As Bill Carr, the guy who refound it says (after finding it 9 paces from where he parked his car on his first stop), 'For me the expe-rience was just another reminder of how few active botanists there are in Texas and how far behind the rest of the country we are in terms of tracking down our rarer resources . . . but maybe that's the point behind the Canon Exploration Grants Program. It provided the stimulus to get a warm body into the field to look for a species that, given the dearth of botanists in this part of the continent, might otherwise have remained enigmatic for who knows how long.' "

Not quite as exciting as the rediscovery of globally "extinct" species, but of potentially equal implications for land management, is the rediscovery of globally rare species that had been considered regionally extinct (i.e., extirpated). Excitement on the Mendocino National Forest in California has centered around the 1996 discovery of several populations of the federally threatened *Howellia aquatica* A. Gray (Lobeliaceae), previously known from California only on the basis of a single fragment collected in 1928 (Isle, 1997). Interpopulational genetic studies are currently under way to compare the California plants with those in Washington, Idaho, and Montana. In addition, another plant that had been thought extinct in California, *Ophioglossum pusillum* Raf. (Ophioglossaceae), was recently located adjacent to one *Howellia* population (D. Isle, pers. comm. 1998).

Even the reappearance of a not-so-rare species in a part of its range where it had seemingly disappeared can be newsworthy, as evidenced by the attention given to a population of *Mimulus tricolor* Lindl. found on the outskirts of Corvallis, Oregon (Holden, 1999). Although this species remained relatively common in the Central Valley of California, it had been assumed to be locally extinct in Oregon. As with *Astragalus agnicidus*, the reappearance of *Mimulus tricolor* after nearly 10 years demonstrates how long a species can persist in the seed bank, and accordingly how difficult it is to verify absence from a site.

(2) Distributional discoveries

More prosaic but gaining significance through sheer weight of numbers is the constant stream of distributional discoveries: major extensions in the known ranges of native species. Only the most dramatic are published (e.g., new state records); the

bulk accumulate in the form of herbarium specimens. A recently verified, curiously overlooked example in the Jepson Herbarium (JEPS) is a specimen of *Luzula piperi* (Coville) M. E. Jones (Juncaceae) from northwestern California *(Ferlatte 349)*, over 600 km south of the nearest previously reported occurrence in northwest Washington (Hitchcock & Cronquist, 1973). A good example from eastern North America is *Schizandra glabra* (E. P. Bicknell) Rehder (Schizandraceae), the only American representative of an otherwise Asiatic genus. A population found in 1991, clambering over a sandstone cliff in southeastern Kentucky, is 250 km from the nearest of the previously known localities scattered across the coastal plain of the southeastern United States (D. D. Taylor, 1994).

More problematic is the recent discovery of *Limnanthes macounii* Trel. (Limnanthaceae) in a seasonally fallow field in west-central California (Buxton & Ornduff, 1998). Previously known only as a rare endemic of southeastern Vancouver Island in Canada, *L. macounii* was at one point presumed extinct (Hitchcock, 1961). What is currently under debate is whether this represents a surprising dispersal event, a previously overlooked natural range disjunction, or evidence that additional populations might exist in intervening sites (A. Ceska, pers. comm. 1998). Ornduff (pers. comm. 1998) supports the dispersal hypothesis, citing the reverse example of *Lasthenia minor* (DC.) Ornduff (Asteraceae) being found in northwestern Washington, over 1000 km north of the nearest naturally occurring population in central California (Vasey et al., 1994). The field in which the California population was found, which was probably significantly larger than the British Columbia population, was subsequently plowed prior to planting cabbage (Buxton & Ornduff, 1998).

Distributional discoveries are not restricted to single species within North America, but can occur as unexpected suites, as evidenced by the following:

- A special category is that of continental-level range extensions: species previously known only from Eurasia that are determined to occur in North America as well, not as introductions but as naturally occurring populations. William Weber (pers. comm. 1998) addressed the large number of Asiatic-Rocky Mountain disjunctions, many recently located, with the comment: "J. D. Hooker was certainly right when he was shocked to see some of his Asiatic things on his five days in the Rockies [in 1877]; sadly, Asa Gray evidently was on a vacation/picnic and didn't recognize that there might be a high latitude component to his Tertiary discoveries in eastern North America."

Continental-level range extensions are not restricted to the arctic and alpine regions, however, but can also be found farther south. For example, specimens from Texas and Arizona previously confused with *Ophioglossum engelmannii* Prantl (Ophioglossaceae) turned out to be conspecific with the widespread Old World species *O. polyphyllum* A. Br. (Zech et al., 1998). More recently, *Eleocharis mamillata* H. Lindb. (Cyperaceae) has been determined to be native and widespread in boreal North America (S. G. Smith & T. Gregor, in prep. 1998).

While continental-level range extensions are noteworthy in vascular plants, they are more routine in bryophytes and lichens (B. Murray, pers. comm. 1998). Even here, however, some examples stand out from the crowd, such as *Aspicilia moenium* (Vainio) Thor. Described in 1986 from Scandinavia, where it often occurs on the mortar of old churches, this lichen was unexpectedly encountered on an old retaining wall connected to the building that houses the COLO herbarium in Boulder, Colorado (Weber, 1996). The account of the discovery of this population amusingly addressed the quandary of how to obtain a decent specimen from an intact structure, solved with the cooperation of Facilities Management staff.

- Conifers are perhaps the best-mapped group of plants in North America (e.g., Little, 1971), being both conspicuous and economically significant. Nevertheless, recent fieldwork by David Charlet has determined that 43% (90) of the 207 conifer-bearing mountain ranges in Nevada harbor at least one more conifer than previously reported, and 12% (24) have had two to four species added to the known complement (Fig. 4). Approaching the same data from a different angle, of the 22 species of conifer known to occur in Nevada, 14 occur on at least one more mountain range than had previously been reported, resulting in 15 new county records in Nevada's 13 western-size counties (Charlet, 1996, pers. comm. 1998).
- On the Pacific Coast, ongoing surveys of nearshore banks that rise to within 30 m of the surface are revealing a hitherto unsuspected and remarkably uniform assemblage of around 40 species of marine macroalgae (seaweeds), extending from Puget Sound to northern Baja California. Included in the assemblage are noteworthy range extensions such as *Pleurophycus gardneri* Setch. & D. A. Saunders (Laminariales), before 1970 recorded only as far south as Oregon but now known to be dominant at depths of 40 m off the central California coast (Kjeldsen, 1972; P. Silva, pers. comm.

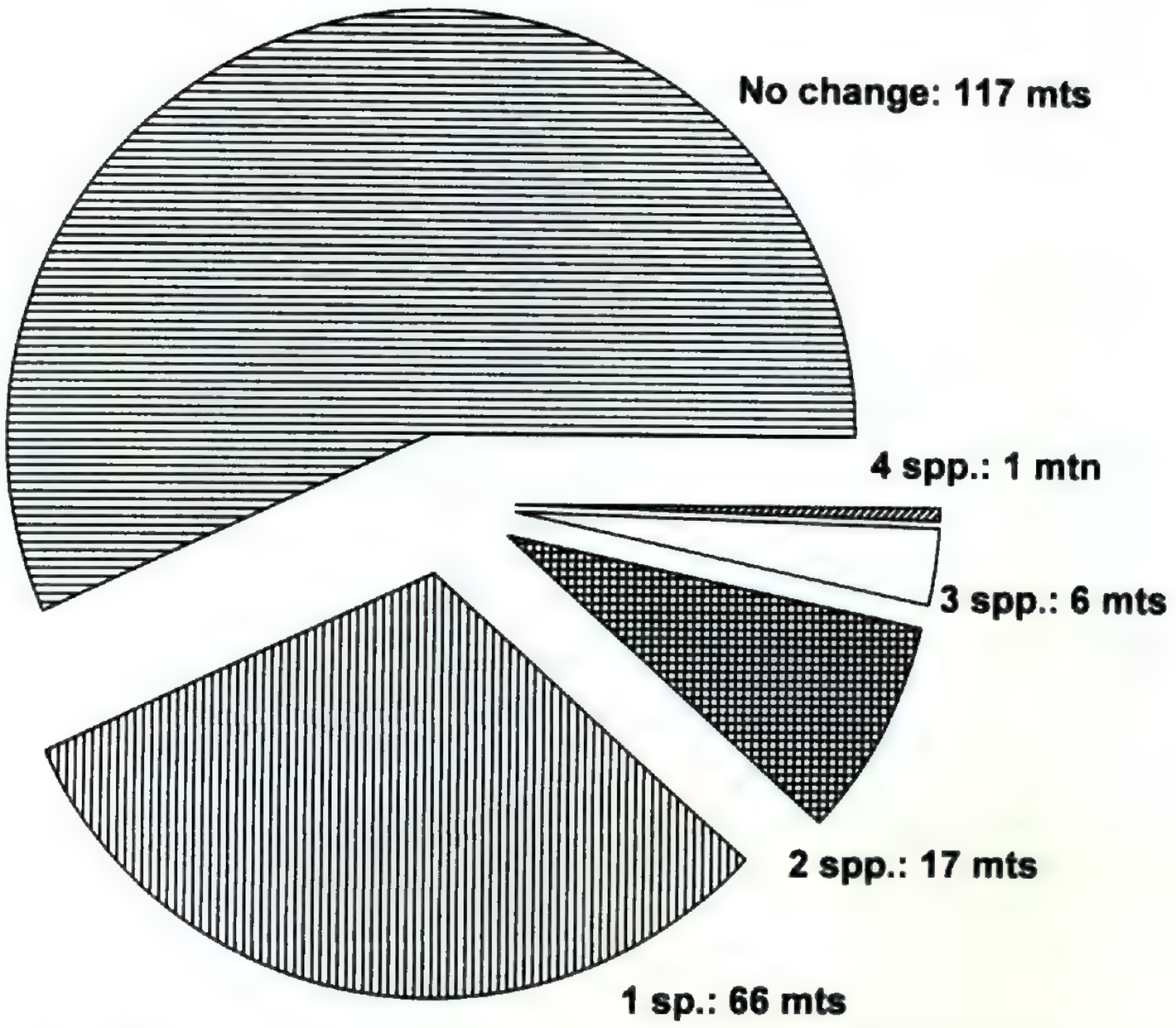

Figure 4. Proportional representation of mountain ranges in Nevada in which additional species of conifers beyond those previously recorded were located during the Charlet survey, 1988–1998 (fig. prepared by D. Charlet).

1998). Although the kelp is readily identifiable, the existence of these southern populations was not apparent because the plants grow at depths that are undisturbed by even violent storms and are accordingly seldom cast ashore.

• Probably the biggest distributional surprise in fungi is the discovery that the occurrence of mushrooms above ground and the fungal species diversity below ground, as determined by molecular analysis of hyphal fragments in the soil, can be completely independent (Gardes & Bruns, 1996). As a result, determining the distribution and rarity of various fungal species presents a challenge well beyond that posed by vascular plants and bryophytes.

(3) Declines and invasions

Whereas all of the preceding examples involve changes to our knowledge of the distributions of species, there are also actual changes in the distributions themselves. On the one hand are significantly diminished ranges, in which historical occurrences documented by herbarium vouchers no longer reflect current distributions. An example is afforded by *Horkelia cuneata* Lindl. subsp. *puberula* (Greene) D. D. Keck (Rosaceae), in which a significant portion of the historically documented range has disappeared under Greater Los Angeles (Ertter, 1995). This kind of distributional attrition has obvious conservation implications, but is extremely difficult to become cognizant of, such that it is theoretically possible for a species to go extinct before its endangered status has even been noted.

On the flip side, and often contributing to the decline of native species, is the spread of species into areas where they did not historically occur. The explosion of aggressive non-natives is of increasing concern due to the various negative impacts such invasions can have on both natural and economic systems. The sheer numbers of newly reported non-natives can be mind-boggling, though it is difficult to determine which are new occurrences and which have simply been overlooked, given that naturalized species are historically undercollected. Vincent and Cusick (1998) documented 70 additions to the Ohio flora, and also emphasized the fact that the non-native component of floras is dynamic, with species appearing, flourishing, and occasionally disappearing. Even in the region around the United States' national capital, which has been rather systematically collected since the 1690s, recent surveys have resulted in the discovery of seven new plant records for Maryland (including two native *Carex*), five of which came from the grounds of the Agriculture Research Center in Beltsville (J. Reveal, pers. comm. 1998).

In California, over 70 non-native species are currently known to have become naturalized beyond those included in *The Jepson Manual* (Hickman, 1993; F. Hrusa, pers. comm. 1998). As dramatic

evidence of how difficult such new occurrences are to keep abreast of, at least 19 occur within an hour's drive of the building in which *The Jepson Manual* was edited. Five are even fully naturalized in the Berkeley campus natural areas (*Geranium rotundifolium* L., *Geranium lucidum* L., *Geranium purpureum* Vill., *Hypericum androsaemum* L., and *Hedera helix* L. subsp. *canariensis* (Willd.) Cout.), and at least three others have already achieved significant pest status in local parklands (*Dittrichia graveolens* (L.) W. Greuter, *Limnobium laevigatum* Willd., and *Maytenus boaria* Molina). The dynamic nature of California's non-native flora, as well as the difficulty of obtaining reliable information on current occurrences, has been addressed by Rejmanek and Randall (1994).

E. WHY NOT FOUND BEFORE?

The preceding examples should serve to emphasize that the era of significant floristic discoveries in North America north of Mexico is far from over, despite perceptions extending back to the mid-19th century. Even the initial cataloging of novelties is incomplete, to the extent that conspicuous shrubs along highways are still being discovered and described as distinctive new species. The comprehensive mapping of known species, including newly invasive pest plants, is equally erratic, at a time when such information is sorely needed to make sound science-based land-management decisions.

The inevitable question arises as to *why* so much of our floristic heritage has remained unexplored, uncataloged, and unmapped. The principal answer is relatively straightforward: it's a big job! As a result, and as many of the previous examples testify, a primary factor contributing to ongoing floristic discoveries is the number of people who are actively scouring the field. Fortunately, this is by no means limited to professional scientists in academic institutions, but instead depends heavily on the collective efforts of agency biologists, environmental consultants, and native plant enthusiasts (Ertter, 1995; Yatskievych, 1999). Representatives of this diverse group are highlighted in a later section of this paper.

However, the number of people actively searching is only one aspect of floristic discoveries. The remaining portion of this paper accordingly addresses other assumptions that have influenced the cataloging of North American plants, both historically and currently.

Assumption 2: Hypothesis-Free Descriptive Science

One key assumption is the common and recurring one that taxonomy and floristics, as examples of "descriptive" science, are not intrinsically scientific, at least as contrasted to the more overtly experimental sciences. This assumption has directly influenced hiring, funding, and promotional decisions, which in turn determines research priorities. Although the full structure of my argument is beyond the scope of the current paper, I will nevertheless posit that science is most definitely involved in all aspects of taxonomy and floristics, complete with the full panoply of falsifiable hypotheses and scientific methodology, even when these are not explicitly expressed.

A. DESCRIPTIVE HYPOTHESES

The assumption that recognizing and "describing" novelties is a simple descriptive process reflects an outdated understanding of biodiversity as consisting of discrete, pre-Darwinian quanta, lacking significant internal variation and separated from one another by inviolate boundaries (Ertter, 1997a). This was noted by Constance (1971: 22) over a quarter-century ago: "Although the doctrine of 'Special Creation' of species has lacked any scientific status for a hundred years, many people seem still to be thinking in terms of a finite number of objects created once and for all, and which merely have to be recognized, described, and named."

Much of the confusion has a semantic underpinning, in that "describing" a species is by no means equivalent to "describing" a concrete individual item. To place species "description" in the explicit framework of set theory and hypothesis generation, the standard phrase:

> "A description of new species *Alpha beta*, which differs from other species of *Alpha* in characters X, Y, and Z"

can be expanded into the complex hypothesis:

> "There exists a previously undiscerned component of natural diversity that falls within the biological parameters of the current species concept, which is hereby coded as species-set *Alpha beta*. As both support for and corollary of this hypothesis, all members of species-set *Alpha beta* are hypothesized to possess biological attributes X, Y, and Z, whereas all members of other species-sets in genus-set *Alpha* are hypothesized to lack this combination of biological attributes."

Furthermore, not only are species and their circumscriptions best understood as complex hypotheses, but so also are such seemingly "factual" statements as "Leaves (2.4)3–5(6.1) cm long," which is in actuality shorthand for the predictive statement: "Based on a measured subset, leaf-length for ALL leaves for ALL members of species-set *Alpha beta*, past, present, and future, is predicted to be at least

2.4 cm long but no more than 6.1 cm long, with the majority falling in the 3–5 cm range."

In both cases, the first phrase is obviously much less cumbersome, but the expanded version more clearly expresses the fact that nested hypotheses are involved, all of which are subject to subsequent testing and modification whenever new data are obtained, most often in the form of new collections of plants that "haven't read the book." Even the identification of an individual specimen can be worded to reflect the complexities of set-assignment, to wit: "The specimen in hand possesses the diagnostic biological attributes that characterize members of the set *Alpha beta.*"

B. PARSING SPECIES

In other words, rather than being routinely simple and straightforward, the task of parsing biodiversity into taxonomic components can be a significant intellectual challenge. As a result, although blatantly distinct species are still being encountered, the majority of recently described novelties are determined to be such only after an extended and detailed comparison with other species, often requiring a wholesale re-thinking of existing taxonomic frameworks. In these cases, it is not at all intuitively obvious what qualifies as a "previously undiscerned component of natural diversity" that falls within "the biological parameters of the current species concept," based on some yet-to-be-determined suite of diagnostic biological attributes.

An excellent example is provided by *Potentilla morefieldii* Ertter (Rosaceae), in which multiple collections had accumulated and been variously identified as (= assigned to species-sets) *P. pseudosericea* Rydb., *P. pensylvanica* L., or *P. breweri* S. Watson (Ertter, 1992). The non-obvious nature of the taxonomic hypothesis that an undescribed species was involved is evident from the fact that an earlier numerical analysis of phenetic variation failed to uncover the novelty (Johnston, 1980). The species is actually quite distinct, once the appropriate diagnostic attributes are highlighted.

In fact, it is more the norm than the exception for the first few collections of a species to be shoehorned into existing species-sets, generally with modifications to the "biological attributes" hypotheses. Shevock and Taylor (1987), for example, noted a range of 1 to 121 years between earliest herbarium specimen and publication in their analysis of California novelties, with an average of 41 years! An even greater span is noted by Hartman and Nelson (1998), with the oldest holotype over 200 years old, and 60% of the novelties having type specimens over 10 years old. However, Hartman's and Nelson's statistics underrepresent the actual range between initial collection and date of publication, in that the earliest collection is not always chosen as holotype. *Monardella beneolens* Shevock, Ertter & Jokerst (Lamiaceae), for example, was typified on a 1986 collection *(Shevock, Bartel & York 11727)*, but included among the paratypes was an 1896 collection *(Purpus 1866)* that had languished in the undetermined-to-species folder for nearly a century (Shevock et al., 1989). This example also illustrates that the distinction between a novelty based on a new discovery and one resulting from a novel analysis of existing specimens is not always clear-cut, in that the collection of an undescribed *Monardella* on a 1986 "Inter-Institutional Haybaling Expedition" is what triggered the herbarium search that uncovered the older specimen.

Nor does the proposing, testing, and rejection of alternate hypotheses end once new species are described. For my doctoral work, I essentially tested the hypothesis proposed by my advisor that a series of annual *Juncus* (Juncaceae) did not meet the criteria for recognition as distinct species, as had been previously proposed (Hermann, 1948), but rather "appear to be mere technical variants, often locally constant as in self-pollinated groups in other genera, but with widely overlapping ranges and similar habitat requirements" (Cronquist, 1977: 64). As it turned out, my doctoral work not only provided support for all of Hermann's hypothesized species except one, but gave evidence of three additional novelties (Ertter, 1986). Although I enjoy the notoriety that comes with being able to say that I proved Art Cronquist wrong *and got him to admit it,* I will also submit that his was a perfectly legitimate hypothesis *based on the information available to him at the time.*

C. FLORISTIC MODELS

The last example introduces the concept of monographs and floras as representing complex models encompassing multiple species, whose individual identities depend on the larger context. As a result, the binomial *Juncus kelloggii* Engelm. codes for three very different entities, depending on whether it is in the context of Hermann's, Cronquist's, or Ertter's model. In this example, sufficient evidence has been accumulated to support one model over the alternatives, but this is not always the case. A contrasting example is presented in Table 1, a partial list of corresponding units of the taxonomically challenging genus *Po-*

Table 1. Concordance of selected species from alternate taxonomic models of *Potentilla* (Rosaceae) in the Intermountain West (= portions of Arizona, California, Idaho, Nevada, Utah, and Wyoming), as proposed by contemporaneous authors: N. H. Holmgren (1997), B. Ertter (treatment for *Flora of North America*, in prep.), J. Soják (unpublished 1995 synopsis of North American tribe Potentilleae), and S. L. Welsh (1993; Utah only, "n/a" indicates entities not occurring in Utah). Table includes some unpublished combinations used by Soják.

Holmgren (1997)	Ertter (in prep.)	Soják (unpublished)	Welsh (1993)
Potentilla pensylvanica	Potentilla pensylvanica var. strigosa	Potentilla pensylvanica var. pensylvanica	Potentilla pensylvanica var. pensylvanica
Potentilla bipinnatifida (= pensylvanica)	Potentilla bipinnatifida var. bipinnatifida var. ovium	Potentilla litoralis var. litoralis var. ovium	
Potentilla pseudosericea (= rubricaulis)	Potentilla pseudosericea [to be determined]	P. hookeriana var. hookeriana var. paucijuga	(pseudosericea n/a) (= rubricaulis) var. paucijuga
Potentilla rubricaulis	[to be determined]	P. rubricaulis	Potentilla rubricaulis
Potentilla concinna (= rubricaulis) (= concinna)	Potentilla concinna var. concinna [to be determined] (= concinna)	Potentilla concinna var. concinna ? Potentilla × concinnaeformis var. concinnaeformis var. beanii	Potentilla concinna n/a var. modesta var. proxima
Potentilla bicrenata	Potentilla bicrenata	P. concinna var. bicrenata	var. bicrenata
Potentilla diversifolia var. diversifolia (= concinna) (= diversifolia)	Potentilla diversifolia Potentilla glaucophylla	Potentilla × diversifolia var. diversifolia var. proxima Potentilla glaucophylla var. glaucophylla	Potentilla diversifolia var. diversifolia P. concinna var. proxima
var. perdissecta	[to be determined]	var. perdissecta	n/a
Potentilla gracilis var. fastigiata	Potentilla gracilis var. fastigiata	Potentilla fastigiata var. fastigiata var. hallii var. jucunda	Potentilla gracilis var. glabrata
	var. permollis	var. permollis	
	var. brunnescens	Potentilla nuttallii	var. brunnescens
var. flabelliformis	var. flabelliformis	Potentilla flabelliformis	n/a
var. elmeri	var. elmeri	Potentilla pectinisecta var. pectinisecta var. comosa	var. elmeri
var. pulcherrima	Potentilla pulcherrima	Potentilla × pulcherrima var. pulcherrima var. wardii Potentilla filipes var. filipes Potentilla × lupina	var. pulcherrima

tentilla (Rosaceae) occurring in the Intermountain West, as proposed by four different specialists, all with access to the same data. The lack of consensus is not an indication of an inability to agree on standards, of taxonomists not being able to "get their acts together," but is rather a reflection of four equally valid models for which insufficient evidence currently exists to strongly support one over the others.

An even larger-scale example of a floristic model is provided by the numerous differences between the comprehensive list of California taxa as summarized in *The Jepson Manual* (Hickman, 1993) and the contemporaneous *Inventory of Rare and Endangered Plants of California* (Skinner & Pavlik, 1994). As analyzed by Skinner and Ertter (1993), the differences result not from one or the other being intrinsically "wrong," but from legitimate philosophical differences in the rationales behind the two publications. The goal of the *Manual* was to maximize the likelihood of unequivocal identification, while that of the *Inventory* was to highlight

units of plant diversity that merited conservation attention. These different goals resulted in different models; in those situations where there was legitimate room for alternate taxonomic hypotheses, the *Manual* tended to lump where the *Inventory* tended to split, so as to avoid "lamentation over taxa that are shown to be distinct only after their disappearance" (Skinner & Ertter, 1993: 27).

D. NOVELTIES IN WAITING

Nevertheless, even within an *Inventory*-type model emphasizing the smallest defensible units as worthy of taxonomic recognition, the requirements for scientifically legitimate, peer-reviewed publication of novelties demand rigorous support for the proposed taxonomic hypothesis. For example, the *Draba* study by Windham and Beilstein (1998a, b), discussed in an earlier section, clearly demonstrates how sophisticated an analysis is often required even for the recognition of unequivocally distinct species. In addition, although many of the highlighted novelties prove that radically different species are still being discovered, the truth is that the majority of blatantly distinct and/or readily encountered taxa have already been described. As a result, ferreting out the remainder will require not only continued exploration, but also increasingly rigorous scientific analysis.

Because of this, there currently exists an unknown number (50? 200? 500?) of potential novelties from North America that members of the taxonomic community are collectively aware of, but which need to be extensively tested before being written up for publication. I am personally aware of several possibilities, in *Juncus*, *Rosa*, *Potentilla*, *Horkelia*, *Eriogonum*, *Montia*, and *Lomatium*, and in fact have as a rule of thumb that any complex group that has not been intensively monographed recently is likely to harbor undescribed novelties. However, all of these possibilities are just that, possibilities, and will require a significant investment of research effort to determine if they are rigorously supportable as taxonomic hypotheses. In other words, the limiting factor for many novelties is not whether they have been encountered or not, but the existence of persons with sufficient expertise, motivation, and time to undertake the necessary scientific analysis.

Assumption 3: Academic Participation

To recapitulate, although the initial discovery of novelties does not require professional training, the analysis of potential novelties is another matter, in which scientific expertise plays a crucial role. Most collectors accordingly rely on the network of taxonomic specialists, who in turn rely on the analytical resources represented by herbaria and botanical libraries, as well as established and innovative technologies. These resources, along with the custodianship and transmission of the extensive legacy of taxonomic knowledge, skills, and techniques, have traditionally fallen within the domain of plant taxonomy in an academic setting, including research museums and botanical gardens.

A. THE ROLE OF REGIONAL FACULTY

Although university-based faculty are only one category of professional expertise (versus, for example, research staff and museum-based professionals), they are highlighted here on the grounds that they are generally assumed to provide the backbone of the rigorous taxonomic analysis described in the previous section, especially faculty at those universities with large herbaria that occur in the regions where most novelties are being discovered. In this context, it is illuminating to analyze the current status of persons in table 4 in Hartman and Nelson (1998): "Individuals who authored six or more novelties of North American plants during the past two decades," according to the categories in Table 2. Of the 56 individuals listed, the two largest categories, both in number of individuals and number of novelties, are "Emeritus (or nearly so)" and "Deceased." Together, the two categories account for 60% of the novelties described from 1975 through 1994. In contrast, faculty who are currently mid-career account for only 6% of the novelties.

To pursue the specific question of novelty description by regionally based faculty further, I polled plant systematists at universities who matched all of the following criteria:

(1) Located in the contiguous western United States (Arizona, California, Colorado, Idaho, Montana, Nevada, New Mexico, Oregon, Texas, Utah, Washington, Wyoming), a region with a high rate of ongoing discoveries.
(2) Located at a university or college with an herbarium of at least 20,000 specimens, representing the equivalent of a fully equipped laboratory for doing taxonomic research on the local flora.
(3) Self-defined as vascular plant systematist (versus ecologist, plant population geneticist, etc.) OR serving as director/curator of the departmental herbarium.
(4) Department-based (versus adjunct) faculty appointment, excluding emeriti, as those persons

Table 2. Current status of individuals listed in table 4 of Hartman and Nelson (1998), "Authors of 6 or More Vascular Plant Taxa North America North of Mexico from 1975 through 1994." The first column gives the total number of individuals in each category, and the second column is the sum total of novelties described by these individuals. Authors describing fewer than 6 novelties are not included in the tally, nor are novelties described by these individuals.

Current status	No. (percent) of persons	No. (percent) of novelties
Deceased	13 (23%)	174 (21.4%)
Faculty		
Emeritus (or within several years)	12 (21%)	316 (38.8%)
Early to mid-career	5 (9%)	50 (6.1%)
Non-faculty academic staff	5 (9%)	65 (8.0%)
Museum staff	9 (16%)	81 (9.95%)
Government agency biologist	4 (7%)	46 (5.65%)
Environmental consultant	2 (4%)	29 (3.6%)
Private individual	2 (4%)	22 (2.7%)
Unknown	4 (7%)	31 (3.8%)
Total	56	814

whose hiring, promotion, and tenure are determined by current departmental expectations.

The specific question addressed was whether each respondent had described (a) zero, (b) one, or (c) more than one vascular plant novelty from anywhere within the contiguous western United States. The number of responses to this survey was gratifying, but the collated results (Table 3) are thought-provoking. Of the 56 persons included in the survey, over half had not described a single novelty from the region, and over half of the remainder had only described a single novelty (or at least had one in press). In several cases, this solitary western novelty was described during the course of graduate work but not since attaining faculty status. Only 10 qualifying faculty members in the entire region have described more than one novelty from the region, and several of these persons are within a handspan of years from retiring.

Furthermore, of the 48 western universities with significant herbaria, 5 currently lack faculty-level vascular plant systematists, including 2 that house the largest herbaria for their respective states (University of Montana, Missoula; University of Nevada, Reno). In Oregon, the two primary herbaria were recently combined, eliminating the position of plant systematist at the University of Oregon, Eugene. One state (Colorado) currently lacks a faculty-level plant systematist who has published any novelties from the region, while four others (Arizona, Montana, Nevada, Washington) can claim only one faculty systematist who has described a single novelty from the target area. This in a region in which 813 taxa were described from 1975 through 1994, around 41 per year, with no evidence of tapering off (Hartman & Nelson, 1998).

B. ACADEMIC SELECTION PRESSURES

The purpose of this survey was not to call into question the scientific productivity of the respondents, who are all actively pursuing a commendable diversity of significant research in plant systematics, including describing novelties from other parts of the world. Nor is it intended to slight the significant contributions of individuals outside the admittedly narrow survey criteria, professional and otherwise. The survey does, however, undermine any assumption that faculty-level plant systematists at the best-equipped western universities comprise the major pool of expertise in ongoing efforts to analyze and describe the unknown elements in a novelty-rich regional flora.

Moreover, there is evidence that this is not a statistical curiosity, but rather an indication that the current academic infrastructure actually discourages such participation. Several respondents indicated that they knew of undescribed regional novelties, but could not justify the research time and effort required to publish them. In the words of one such respondent, "the value of new species descriptions in terms of professional prestige and satisfaction of university administrators (who control raises and promotions) seems low relative to other publications that could be generated in a similar

Table 3. Participation of current vascular plant systematist faculty in the contiguous western United States in the description of regional novelties (Ertter, unpublished data). Column 1 is the number of universities or colleges in each state with significant herbaria (defined here as at least 20,000 specimens). Column 2 is the number of department-based (vs. adjunct) vascular plant systematists, and non-systematists actively serving as herbarium director. Columns 3, 4, and 5 are the number of persons in column 2 who have described, respectively, (a) zero, (b) one, or (c) more than one vascular plant novelty from anywhere within the contiguous western United States, including novelties in press. Percent representation among these three categories is given in parenthesis after summary totals. Column 6 is the total number of novelties from each state published 1975 to 1994, tallied in table 8 of Hartman and Nelson (1998).

			Novelties described			Total
	Institutions	Faculty	0	1	>1	novelties
Arizona	3	3	2	1	0	60
California	16	14	8	2	4	223
Colorado	3	3	3	0	0	33
Idaho	3	4	2	2	0	33
Montana	2	1	0	1	0	12
Nevada	2	1	0	1	0	64
New Mexico	2	3	0	2	1	41
Oregon	2	3	1	2	0	44
Texas	8	14	11	1	2	75
Utah	3	3	0	1	2	183
Washington	3	5	4	1	0	13
Wyoming	1	2	0	1	1	32
Totals (percentages)	48	56	31 (55%)	15 (27%)	10 (18%)	813

period of time." In effect, the publication of regional novelties is not only of little value, it is actually counterproductive to career development in the current academic environment. Paradoxically, the fact that the amount of time and effort it takes to publish a novelty can be equivalent to that needed for other research activities in itself provides evidence that describing novelties is not the trivial activity it is routinely perceived to be.

I find it illuminating to compare the preceding quote with another, from nearly a half century ago: "If taxonomy and taxonomists are to regain some of their lost prestige—and they have lost a great deal—it seems obvious that mastery of a local flora, an ability to recognize characteristic members of the more common plant families, a familiarity with the rules of nomenclature, and the capacity to write descriptions are bound to prove woefully inadequate" (Constance, 1951: 229). We have apparently come full circle, where the skills that were once the *sine qua non* of a practicing taxonomist have apparently gone from being "inadequate" to being irrelevant. Or, at best, these former skills are assumed to come as part of the "systematist package," overlooking the tenet otherwise well known to biologists that "you get what you select for." Admittedly, the above analysis is only a single slice in time, but it nevertheless strongly suggests that the activities that result in the publication of regional novelties are NOT among those currently being selected for within academia, and as a consequence are de facto being selected against.

C. WHO IS DOING THE WORK?

The question then arises: If not faculty-level systematists at the best-equipped regional universities, who is responsible for generating the 41 novelties per year in the contiguous western United States? Obviously there are numerous people who are discovering and describing western novelties other than those targeted here, who I have neither surveyed nor otherwise statistically analyzed. The major categories, however, would include the following: emeriti plant systematists; museum-based plant systematists, often with adjunct appointments at nearby universities; faculty-level plant systematists at less well-equipped regional university and colleges (i.e., with herbaria having less than 20,000 specimens); plant systematists outside of the region; non-faculty research and curatorial appointments; non-systematists (e.g., ecologists, population geneticists); government agency biologists; biologists working for the private sector, mostly as environmental consultants; and amateur enthusiasts.

Certainly the academic and museum-based cat-

egories play significant roles, which should not be underestimated. What I wish to draw attention to at this point, however, is the high degree of participation by professionals and amateurs outside of academia, many of whom have an exceptional eye for novelties and a serious commitment to floristic undertakings. As representative examples, some of the more outstanding are spotlighted below:

(1) Government agency biologists

Beginning with his stint in 1979 as botanist for the Sequoia National Forest in California, James R. Shevock has now tallied 6 vascular plants and 1 moss named in his honor, 12 others that he has authored, and several undescribed novelties in various stages of publication. Many of his earlier novelties were encountered by his being the first botanist each year on newly constructed portions of the Pacific Crest trail, which ended up bisecting a population of *Allium shevockii* McNeal that is still one of the only populations known. While retaining a focus on the southern Sierra Nevada, Shevock's botanical interests have subsequently expanded to include mosses and lichens, with *Orthotrichum shevockii* Lewinsky-Haapasaari & D. H. Norris (Orthotrichaceae) being the most recent addition to his eponymous tally. The protologue credits Shevock with "opening the eyes of the junior author to the bryophyte riches of the southern Sierra," a significant accomplishment considering Norris's extensive expertise with the California bryoflora (Lewinsky-Haapasaari & Norris, 1998). Shevock's agency career has likewise expanded; as Regional Botanist, he prepared the status report on rare and endemic plants for the Sierra Nevada Ecosystem Project (Shevock, 1996), and he has recently moved on to become Associate Regional Director of the National Park Service. Shevock's botanical explorations are accordingly now confined to weekends and vacations, but have not noticeably slowed as a result. On a 1996 foray (a.k.a. "death march") to an isolated marble ridge, he and protégé Dana York (botanist for Death Valley National Park) discovered three novelties in a single day: *Heterotheca monarchensis* D. A. York, Shevock & Semple, *Gilia yorkii* Shevock & A. G. Day, and a still-undescribed *Eriogonum.*

(2) Environmental consultants

The tragic death of James D. Jokerst, who drowned while trying to retrieve the family canoe, cut short the career of one of the persons who did the most to convert environmental consulting into a legitimate career for skillful, well-trained botanists. While working full-time for the environmental consulting firm Jones & Stokes Associates, Jokerst nevertheless found time to develop expertise in the Lamiaceae, preparing treatments of several genera in *The Jepson Manual* (Hickman, 1993), including the notoriously difficult *Monardella* (Magney, 1996). He also authored or coauthored three novelties: *Acanthomintha obovata* subsp. *cordata* Jokerst, *Monardella beneolens* Shevock, Ertter & Jokerst, and *Pogogyne floribunda* Jokerst. While doing a botanical survey in 1985, Jokerst discovered an unusual gold-flowered *Trifolium,* which was posthumously named in his honor (Vincent & Morgan, 1998). According to Vincent (pers. comm. 1998), the clover "was found in an area that A. A. Heller had collected at several times, but never early enough in the season!"

Environmental consultants in general are playing an increasingly significant role in discovering novelties, as the persons most likely to have access to poorly botanized areas. As prime examples, the discoveries of *Yermo xanthocephalus, Twisselmannia californica,* and *Neviusia cliftonii* were connected to environmental survey efforts. Unfortunately, a great many biological consultants lack the training or orientation needed to recognize potential novelties, and may in fact be discouraged from taking note of anything but a mechanically generated list of rare species determined to be potentially present at a given site. This practice is based on the dangerously flawed assumption that previously existing knowledge is an accurate indication of likely occurrence, an assumption at odds with the theme of "floristic surprises." As summarized by S. Boyd (pers. comm. 1998), discoverer of *Sibaropsis* and other novelties in the course of doing environmental surveys:

> "There is the strong possibility that other botanical gems are being overlooked by overworked, and sometimes undertrained, botanical consultants too myopically focused on the punchlist of expected sensitive species. It seems to me much better to approach any botanical inventory from the point of 'what is present overall?' vs. 'which sensitive plants are present?' I wonder how many other undescribed taxa have been overlooked and subsequently lost to habitat destruction."

(3) Amateur enthusiasts

Among the more unexpected of the amateur enthusiasts is Lowell Ahart (Geary, 1978), a sheep rancher who started out cataloging the plants of his ranch and has since moved on to county floras, collaborating with retired zoology professor Vern Oswald (e.g., Oswald & Ahart, 1994). Two plants from his ranch have been named after him *(Juncus leio-*

spermus var. *ahartii* Ertter, *Paronychia ahartii* Ertter), and an *Eriogonum* is also being named in his honor (J. Reveal, pers. comm. 1997). When Ahart brought the undescribed *Paronychia* to my attention, begging that someone provide a name for it so he could complete the checklist of his ranch, it had actually been known for some years but had been assumed to represent yet one more introduced Eurasian annual. However, by then a worldwide monograph of the Paronychiinae was available (Chaudhri, 1968), making it evident that an anomalous undescribed species was involved, whose affinities are still unclear (Ertter, 1985).

(4) Other para-academics

A final example of expertise outside of academia is provided by Arnold (Jerry) Tiehm, who has an advanced degree in botany and previous professional experience (e.g., curatorial staff at The New York Botanical Garden). For the last several years, however, Tiehm has earned his living as bell captain and limousine driver at the Peppermill Casino in Reno, Nevada, doing his botanizing on his days off. At last count he has nevertheless made the type collections of 19 species (Holmgren, 1998), approximately one per year, several of which are named after him. Probably the most significant is *Stroganowia tiehmii* Rollins, the single North American representative of a genus otherwise confined to central Asia (Rollins, 1982). It also qualifies as another "in our backyards" discovery, not encountered until 1980 even though occurring only a few miles off a well-traveled highway 20 airmiles east-southeast of Reno.

D. IS THE POOL SUFFICIENT?

These highlighted individuals are only a sampling from a large pool of talented and dedicated individuals operating outside of an academic setting, including some who not only discover but analyze and describe their own novelties. When these para-academics are combined with museum-based systematists, faculty at smaller institutions, and non-faculty research staff, it might accordingly be argued that the existing pool of expertise is sufficient, and that faculty systematists at the larger universities should appropriately be encouraged to address avenues of research that cannot be handled by others.

While confirming my enthusiastic support for a diversity of individual research interests within academia, and likewise for the active participation of individuals outside of the academic mainstream, I will nevertheless argue (as have others, such as Kruckeberg, 1997) that a core of professional plant systematists will continue to play an indispensable role in the task of discovering, analyzing, and describing the remaining unknown element in the North American flora, as well as critically evaluating new information accumulated about previously described species. In other words, rather than being made redundant by the para-academic network, an active core of professional systematists is integral to the proper functioning of the network. Furthermore, a significant percentage of this professional core needs to be housed at the large regional herbaria, especially in the West and Southeast where the majority of floristic discovery is occurring.

The obvious argument for academic participation is, of course, to provide the formal systematic training for all other participants in the network, including agency biologists and environmental consultants. Perhaps even more critical, however, is the reality that regionally based professional systematists represent the essential source of quality control and accessible scientific expertise to turn to when non-systematists encounter "plants that haven't read the book." Furthermore, para-academics who analyze and describe their own novelties generally do so only after a period of "apprenticeship" with a regionally based, practicing taxonomist. In this regard, it is unsettling to realize how many of the regional professionals who provided early encouragement and training to the current crop of active para-academics are now retired or deceased. A prime example of the latter is the late John Thomas Howell of the California Academy of Sciences, who provided significant encouragement to most of the individuals highlighted above.

Howell also represents the category of museum-based research staff that is becoming increasingly important in maintaining the role of the professional core. As significant as this contribution is, however, museums and botanical gardens are too few in number to provide complete regional coverage, and are also less likely to be involved in formal training. At the same time, the suggestion that descriptive systematics should be relegated to smaller universities and colleges runs counter to the fact that the major herbaria are generally at the larger universities. The continued participation by major regional universities is therefore essential to the ongoing task of discovering and analyzing the regional flora. If this task is in fact incompatible with academic realities currently facing faculty systematists (and not just a matter of erroneous perceptions), then it is imperative that alternate ways to ensure such participation be investigated, perhaps involv-

ing direct funding or collaborative programs with state or federal land-management agencies.

E. THE TAXONOMIC LEGACY

Approaching the situation from a different angle, there is the question of the taxonomic legacy, a term used here to refer to the material resources (i.e., regional herbaria and associated libraries); the fundamental knowledge, skills, and techniques for floristic analysis; and the setting of scientific standards, generally in a peer-reviewed context. Custodianship of this legacy, developed over several centuries, has traditionally fallen within the domain of plant taxonomy in an academic setting: universities, research museums, and botanical gardens. The question that needs to be asked is not only to what extent this legacy is being maintained, but to what extent the components are currently available to whomever is actually doing the bulk of analyzing and describing regional novelties, whatever their professional credentials and self-identities might be.

A prime example is the situation referred to above, in which large universities that house major herbaria struggle to justify their upkeep while simultaneously relegating descriptive systematics to less well-equipped or well-situated institutions. The transfer of floristic survey work to the domain of ecologists, another apparent trend, is also a potential problem if it is not accompanied by the transfer of associated skills and techniques (e.g., critical taxonomic analysis and an understanding of the role played by vouchers). Paradoxically, it is possible that, in the name of increasing the scientific respectability of systematics, one outcome might actually be a net *decrease* in the scientific standards underlying the analysis and description of new species, the very foundation of our knowledge of biodiversity.

Assumption 4: The Property Rights Conflict

A. SOCIOLOGICAL RAMIFICATIONS OF FLORISTIC SURPRISES

Furthermore, what are the broader ramifications of allowing taxonomy and floristics to take place largely as a collective avocation, a labor of love even for those in professional positions, rather than an academically supported undertaking? For better or worse, the days when new taxonomic and floristic discoveries were of concern only to professional botanists and amateur enthusiasts are far behind us. Although there are numerous exceptions (e.g., *Juncus tiehmii* Ertter, which is widespread in western North America), the majority of newly described species qualify for some level of sensitive species status, with immediate implications for land-management activities on public and private lands. Problems can arise if negatively affected landowners develop the impression that so-called "amateurs" are behind newly described rare species without the backing of socially sanctioned expertise.

Even for those species that aren't novelties, a lack of sufficient information on their taxonomy and distribution interferes with effective conservation efforts. The magnitude of this problem can be seen in the list generated by Skinner et al. (1995) of 182 rare California plants for which further taxonomic resolution is needed, and another 44 that require additional distributional information before their conservation status can be properly assessed. The implications of this knowledge gap become apparent in the face of decisions that are being made now which will irrevocably determine the fate of much of our natural heritage, representing a vast resource containing both pragmatic and aesthetic values.

B. IN FRONT OF THE BULLDOZER

In this context, it is unsettling to realize how many plants (and other organisms) that would qualify for some level of management activity, possibly representing 5% of the North American flora (based on Taylor's extrapolations as previously discussed) and including some of the rarest of the rare, are currently receiving NO attention because they have not yet been discovered, analyzed, and described. In other words, we risk losing a significant percentage of our floristic heritage out of sheer ignorance of its existence, not just in the tropics but in our own backyards.

In support of this statement, an increasing number of novelties in North America, as in other parts of the world, are being discovered "in front of the bulldozer." The type locality of *Neviusia cliftonii*, for example, is threatened by a limestone quarry, and the monotypic genus *Yermo* was discovered as part of a survey along a proposed pipeline route. The narrow endemic *Ivesia aperta* (J. T. Howell) Munz var. *canina* Ertter (Rosaceae) was still in manuscript when plans to build a dam that would have flooded almost the entire population came to light (Ertter, 1988). *Ceanothus ophiochilus* S. Boyd, T. S. Ross & L. Arnseth (Rhamnaceae) was found during the environmental impact study of a proposed development in southern California (Boyd et al., 1991). Even more recently, the type population

of an undescribed *Pseudostellaria* (R. Hartman & R. Rabeler, in. prep.) was found adjacent to the staging area for an active logging site, potentially surveyed for sensitive species prior to approval for timber harvest, but not for undescribed taxa. A new tarweed currently being described from Livermore, California, also falls into this category, occurring as it does in an area of some of the heaviest development pressure in the San Francisco Bay Area (Baldwin, 1999).

Unfortunately, there is also evidence of potential novelties being eliminated before they could even be described. This may be the case with an undescribed *Eriogonum* mentioned in the protologue of *E. capistratum* Reveal var. *welshii* Reveal (Reveal, 1989b), whose only known population in southwestern Idaho has possibly been eliminated by the construction of communication towers.

C. LANDOWNER RESISTANCE TO SURVEYS

In-front-of-the-bulldozer discoveries, exciting as they might be to the botanical community, can be a decidedly rude surprise for the landowner, representing an unexpected and potentially very expensive complication in what might otherwise have been a relatively straight-forward and profitable undertaking. One newspaper covering the rediscovery of the Ventura Marsh milkvetch *(Astragalus pycnostachyus* var. *lanosissimus)* noted how the plant was "causing trouble" and had "thrown a kink" in the developer's plans to build $300,000 homes surrounding a man-made lake *(Sacramento Bee,* 15 Aug. 1997). Fanned by negative publicity and property rights advocates, the fear that property values and development options will be severely curtailed by the discovery of such unwelcome surprises has unfortunately led to a significant polarization between private landowners and advocates of biodiversity protection. This in turn has often resulted in a refusal to allow floristic surveys on private lands, which can contain significant portions of relatively unexplored areas that could harbor novelties and populations of other significant plants.

The scale of the fear and distrust has even led to the paradoxical situation in which local landowners insist that "there are thousands" of a so-called rare plant on their properties, while simultaneously refusing to allow the scientific surveys needed to justify less stringent management options. Frank testimony to exactly this situation, and to the massive amount of distrust, fear, and outrage that can build up in the absence of trustworthy sources of reliable information to the contrary, is provided by Janssen and Williamson (1996). In summarizing her efforts to gain access to reported populations of *Frankenia johnstonii* Correll (Frankeniaceae) on the private ranchlands of Zapata County, Texas, state botanist Gena Janssen (in Janssen & Williamson, 1996: 3) shared these insights:

> "As I began to meet and get to know more and more landowners, I began to notice that most of them did basically the same thing when they met me: They yelled at me. And then one day it finally hit me as to why they did that. Finally, they had a person, a warm body, in front of them that represented all these endangered species issues that had been scaring them for so long. They just needed to vent, so I let them. They had no one there for them. There was no one there to say, 'No, that's not true,' or 'Yes, that was a very difficult situation for everyone,' or 'Well, only part of that is true,' etc., until now."

Encouragingly, this stage was the prelude to a particularly noteworthy success story. Janssen's patience, honesty, and willingness to listen compassionately paid off, first in obtaining the access needed to acquire critical distributional and other biological data on a plant that occurs almost exclusively on private land, and subsequently in working with the landowners to develop a voluntary conservation plan. As a result, *F. johnstonii* is currently being removed from the endangered species list, with many of the ranchers now taking legitimate pride in "their" rare plant (Janssen, pers. comm. 1998; see also http://www.tpwd.state.tx.us/news/news/980518a.htm).

In this context, there is a distinct irony in the fact that the fundamental floristic work in Kern County, California, currently a property-rights stronghold, was undertaken by a local rancher, Ernest C. Twisselmann. His contributions to California botany have recently been acknowledged in the form of a newly discovered genus named in his honor, *Twisselmannia* (Al-Shehbaz, 1999). Twisselmann's interest in botany, triggered by an outbreak of nitrate poisoning in his cattle (McClintock, 1973), eventually led to the publication of two floras (Twisselmann, 1956, 1967) and the discovery of several new species (e.g., *Nemacladus twisselmannii* J. T. Howell, *Eriogonum temblorense* J. T. Howell & Twisselm.). The acknowledgments to his 1967 flora provide insight into the respect for private property that lay behind the success of his undertaking.

> "In a time when malicious trespass and vandalism are almost an acceptable form of outdoor recreation, locked gates are essential and a suspicion of even well-intentioned strangers is a natural attitude of rural people. So I am doubly grateful for the open-handed trust and generosity of the many landowners who freely gave me access to their property, and whose friend-

ships have been one of the quite unexpected dividends of the fieldwork."

D. FLORISTIC SURPRISES OR "NO SURPRISES"?

The *Frankenia johnstonii* example is only one among many in which an increased floristic information base, sometimes paid for by the private stakeholders, worked in their favor, either by providing sufficient scientific evidence for reduced protection status (e.g., downlisting) or by increasing the mitigation options. These examples need to be brought together for impact, but currently exist only in scattered documents and word-of-mouth reports. Granted, there is a difference between a ranch family that wishes to continue a way of life requiring large open spaces, and a developer who needs to subdivide and build in order to realize an investment. Even in the latter case, however, the negative consequences of floristic ignorance can cut both ways, increasing the risk of misplaced mitigation efforts as well as the unintentional extinction of species.

As a society, we have acknowledged that the perpetuation of our biodiversity heritage is a highly desirable goal, for pragmatic, aesthetic, and ethical reasons. Within this context, the key question becomes how to accomplish this goal as fairly and effectively as possible. Unfortunately, instead of making the necessary hard decisions on a solid basis of complete scientific knowledge of all elements involved, we are forced to face the tragic fact that the "best available scientific evidence" is often a woefully inadequate reflection of the actual data needed for the kind of far-reaching decisions that are currently mandated.

A common quandary, for example, is determining whether a species is truly as rare as existing evidence indicates. In these circumstances, it is sometimes argued that, if the scientific evidence is incomplete, then no land-management constraints can be justified. This argument, however, runs counter to the fact that all legal decisions, including those addressing environmental issues, are based solely on best evidence available at the time of the decision, with neither hearsay nor supposition having a legitimate role. One can speculate that a species is more widespread than the currently available scientific evidence indicates, but a decision based on this speculation without hard evidence to back it up is no more justified than is ruling on a defendant's guilt strictly on speculation that the person *might* have done the crime. Given this, it is readily apparent that operating from a maximally comprehensive and accurate information base is vastly preferable to acting in ignorance, and willful ignorance becomes inexcusable, if not outright foolish. When all is said and done, the best guarantee of "No Surprises" (the nickname for a key landowner incentive in regional conservation plans) is complete information up front.

ASSUMPTION 5: THE OVERWHELMING CHALLENGE

Within this framework, the significantly preferable option to isolated, development-driven surveys would be a proactive, comprehensive effort to address the existing gaps in our floristic information base. It works to no one's benefit for an undescribed plant or a significant population of a sensitive species to be discovered *after* significant funds have already been expended on a proposed project [e.g., a newly discovered *Draba* that is "complicating Olympics preparations" for the 2002 Winter Games in Utah, having been found at the site of the men's downhill race course (*Deseret News*, 22 Aug. 1998; Windham & Bellstein, 1998b)]. There is furthermore a distinct sense of unfairness in having the short straw fall to the landowner(s) of the last refuge of a once-common species, which only became endangered when neighboring landowners had developed their parcels first.

Avoiding such situations is in fact a primary goal behind the current focus on developing regional conservation plans on which to base land-management decisions. Although excellent in principle, in reality such efforts have often been deficient in addressing species-specific information, in large part because of the assumption that obtaining the relevant species-specific floristic information is too formidable a challenge to pursue. This in turn leads to the argument that alternate information (e.g., satellite imagery, umbrella species) serves as an adequate substitute to field-based, species-specific floristic data. A dramatic counter to this argument is provided by the Shasta snow-wreath (*Neviusia cliftonii*), in which a relatively conspicuous shrub was completely overlooked by one of the most complete vegetation mapping projects ever undertaken (Weislander et al., 1939). As a bottom line, large-scale land-management plans that address only dominant and formally listed species have the potential of allowing the incremental disappearance of all other species in the region, including any undescribed novelties, without even leaving a record of their previous existence.

The challenge of comprehensively addressing the species-specific gaps in the floristic information base is indeed formidable, but the assumption that it is an overwhelmingly unrealistic goal is based in large part on the assumptions previously addressed.

In particular, it can hardly be said that the assumption has ever been put to the test, given the low level of support that floristic efforts have historically received. For such an undertaking to become a reality, however, the following would accordingly need to be addressed:

- acknowledge incompleteness of existing floristic knowledge base;
- assign value to floristic information commensurate with the effort required to generate it and its value to society at large;
- ensure that essential academic resources are available at regional level;
- foster the network of professional and para-professional expertise;
- promote the training and participation of para-professionals within a framework of acceptable scientific standards;
- depolarize relations with private landowners, with academic participation providing an essential agenda-neutral framework;
- disperse floristic information in a framework that addresses the particular needs of all participants.

Several possible prototypes incorporating one or more of these elements have already been developed. The Rocky Mountain Flora Project, for example, demonstrates the scale that can be accomplished by focused floristic surveys within an academic setting (Hartman, 1993). In contrast, the Oregon Flora Project depends less on graduate student projects and more on existing and newly generated information from an extensive network of academic, agency, and native plant society sources, critically analyzed by herbarium-based professional systematists (Sundberg, 1997). The 1980 peak of novelty description in Utah, as shown in Figure 1, resulted in large part from the collaborative activities of regional academics, agency biologists, and environmental consultants, and similarly collaborative "haybaling expeditions" have taken place in Idaho (Big Horn Crags) and the southern Sierra Nevada. In the San Francisco Bay area, a regional checklist was specifically designed to facilitate and encourage the participation of para-academics in floristic inventory efforts (Ertter, 1997b). In that all of these efforts, and the discovery of "floristic surprises" in general, have proceeded with minimal institutional support in an increasingly avocational network, one can only speculate as to what could potentially be accomplished within the framework of a well-coordinated, seriously supported floristic undertaking, taking full advantage of both professional and para-academic networks.

The Big Picture

In conclusion, I propose that what taxonomists have been up to is nothing less than one of the most massive scientific endeavors ever undertaken: namely, a centuries-long, internationally collaborative effort to model global biodiversity. If this does not qualify as "Big Science," I don't know what does! The significance of this undertaking takes multiple forms, starting with the fundamental desire to know what other forms of life share this planet with us, the only island of life we know for certain exists in the universe. The resultant model also forms the foundation underlying other branches of biological knowledge, and it follows that the more complete and accurate the model is, the stronger the foundation is (cf. the "taxonomic impediment" of R. W. Taylor, 1983). Most important, as we now find ourselves in an era when crucial decisions are being made that will determine the face of life on the planet, it is imperative that these decisions be made with the most comprehensive information possible.

Furthermore, the challenge of obtaining the species-specific floristic information needed to make science-based land-management decisions in North America north of Mexico, although formidable, is not beyond our grasp. However, the viability of the essential professional taxonomic infrastructure needs to be ensured, and the undertaking approached as a seriously supported collaborative effort combining academic and para-academic resources at the regional level. If not, then we risk losing 5% of the floristic diversity in the North American "backyard" by ignorance alone, as well as unfairly allocating the conservation costs for biodiversity in general.

A quote by Thomas Bridges opened this paper, expressing his amazement that there were still floristic surprises in North America in 1858. I will end with a more accurate perception by Dieter Wilken, expressing his delight in the Colorado flora in 1984, over a century after Bridges' visit to California (transmitted by R. Patterson, pers. comm. 1998): "I am continually amazed at the things that are yet to be discovered."

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A NEW AGE OF DISCOVERY[1]

Michael J. Donoghue[2] and William S. Alverson[2,3]

ABSTRACT

Whereas past ages of discovery have been associated with the exploration of new continents, the current episode is marked by exploration at smaller spatial scales and in more extreme habitats, some of which are surprisingly close at hand. This new age of discovery is richer and more illuminating by virtue of the attention being paid to phylogenetic relationships and geographic ranges. Although it is difficult to quantify progress in understanding the tree of life or biogeographic patterns, a series of examples illustrates that the magnitude and significance of recent discoveries are unquestionably great. Extraordinary discoveries provide an obvious, yet underutilized, mechanism to capture the imagination of the scientific community and the public at large.

Key words: age of discovery, biodiversity, biogeography, exploration, new species, phylogeny.

We tend to regard "the age of discovery" as something long since past. Whether the phrase refers to the colonization of North America by trans-Beringian migrants, arrival of the Moa Hunters and Maoris in New Zealand, or (most likely) the forays of European explorers into the New World from the 15th century onward, depends on one's sense of history and focus. However, there may be general attributes of all previous epochs with which we can evaluate whether any particular period really qualifies as an age of discovery. We will argue that the present era most certainly deserves this title.

Briefly consider the most recent of these ages of discovery, which began in the late 1400s. European exploration of the Americas, Australia, Africa, and the South Pacific brought to light an extraordinary number of new species. Even more importantly, these were species quite unlike any known before: the rhinocerous (Fig. 1), the kangaroo, and the dodo, to name only a few. David Quammen (1996) helps us imagine what it must have been like to hear about or see such animals for the first time:

> ". . . the first report of a hummingbird, . . . of a toucan, . . . of giant anteaters and sloths and vicunas . . . first report of the American bison, a giant-headed animal almost too majestic to be real. Imagine how these prodigies must have unnerved a complacently pious sixteenth-century European."

How can the present day possibly compare either in terms of the number of novelties being discovered or their "surprisingness"?

NEW SPECIES

In most major lineages the number of new species being described is not falling off. Instead, the numbers have actually been rising over the last decade to levels comparable to those of the mid-18th to late 19th century (before which comparisons are difficult, since formal rules for naming species had not been developed). Surprisingly, perhaps, this includes the groups that we tend to think of as being the best known. For example, the description of new mammal species is on the rise (Wilson & Reeder, 1993), and this is not, as one might initially suppose, primarily due to the splitting of known species as a consequence of using molecular markers. Instead, it appears to be directly correlated with serious exploration of new areas (Morell, 1996). For instance, fieldwork over the last decade in the Annamite mountains on the border of central Vietnam and Laos has brought to light several large

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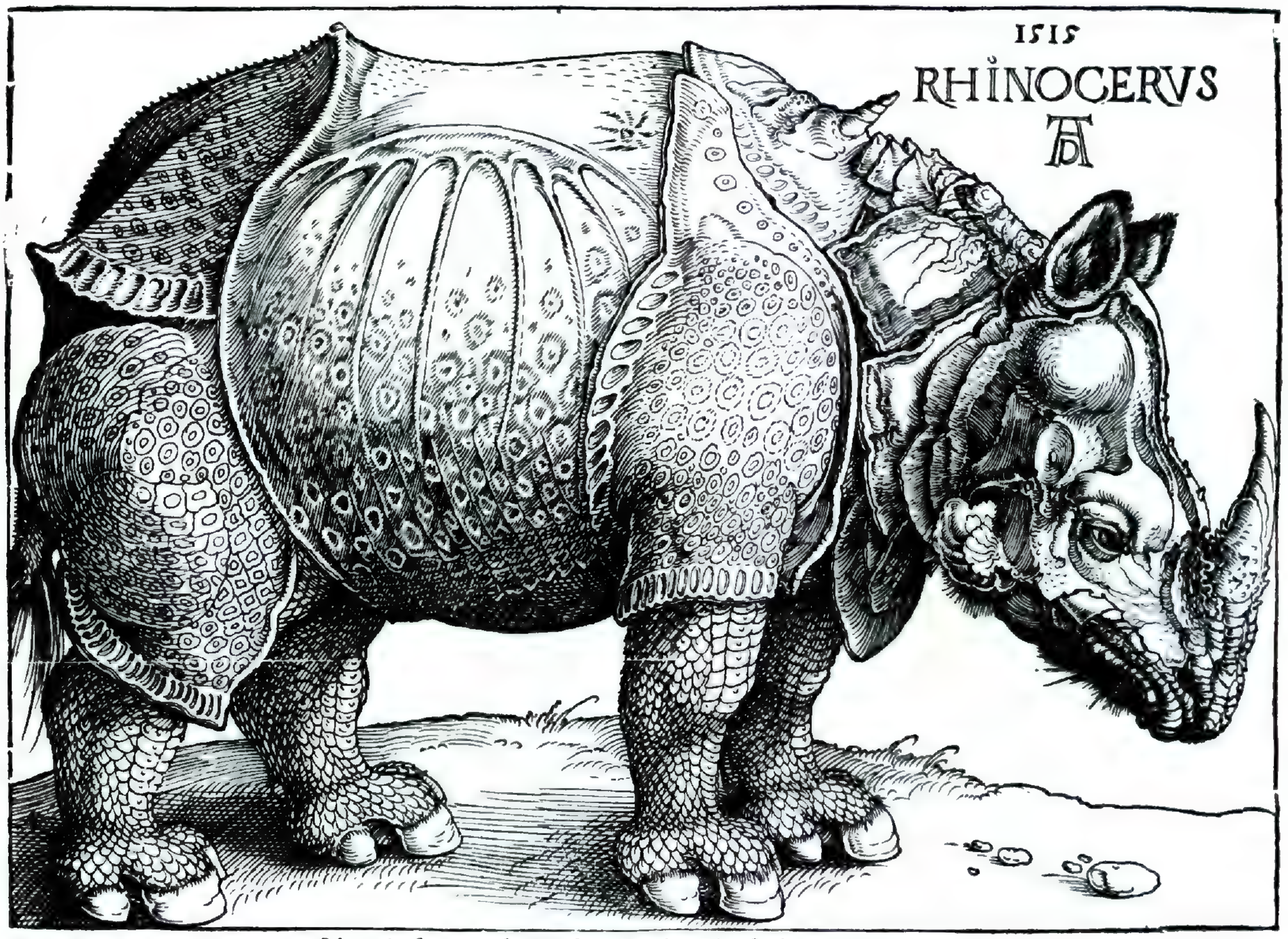

Figure 1. The fantastic rhinoceros, as conceived by Albrecht Dürer at the time these animals were first discovered by Europeans. [Reprinted, with permission, from an original print at the Agnes Mongan Center, Fogg Art Museum, Harvard University Art Museums.] Nearly two centuries later, upon seeing a rhinocerous, John Evelyn remarked, "It more ressembled a huge enormous Swine, than any other Beast amongst us . . . but in my opinion nothing was so extravagant as the Skin of the beast, which hung downe on her ha[u]nches . . . loose like so much Coach leather . . . and these lappets of stiff skin, began to be studdied with impenetrable Scales, like a Target of coate of maile, loricated like Armor . . . . T'was certainly a very wonderful creature, of immense strength in the neck and nose especially" (Evelyn, 1684).

mammals—a species of bovid, *Pseudoryx nghetinhensis* (Dung et al., 1993), and at least three species of muntjac deer (Schaller & Vrba, 1996; Giao et al., 1998; MacKinnon, 2000 this issue). Moreover, the rate of description of new mammal species has risen despite decreased attention to taxonomy and a documented increase in the time between discovery and description (Patterson, 1994).

Amphibians provide another well-documented example of the rising rate of species description (Glaw & Köhler, 1998; see Fig. 2). Again, this reflects, to a considerable extent, the discovery of novelties in the wild, rather than the application of molecular methods or changes in species concepts. Hanken (1999) documented this point, citing David Wake's (1996) discovery of a new lungless salamander from the San Gabriel mountains, less than 30 miles from Los Angeles, as well as species described in 1998 from Brazil, Mexico, Nepal, and New Zealand.

The same trends in discovery (if not formal naming) doubtlessly apply in less well studied groups, such as plants, fishes, insects, mites, nematodes, and especially Bacteria (eubacteria) and Archaea (archaebacteria, e.g., Pace, 1997; Fuhrman & Campbell, 1998; Madigan, 2000 this issue), although reliable estimates are harder to come by (e.g., see Gaston, 1991, on insects; Prance et al., 2000 this issue, on vascular plants). If there were more taxonomists, and if the lag time between the discovery and naming of new taxa were shorter, then the rate of description would surely be far higher. Many new species have already been collected, or are already known to be new, but remain undescribed simply for lack of expertise and time. Plant monographers, for example, can show any cu-

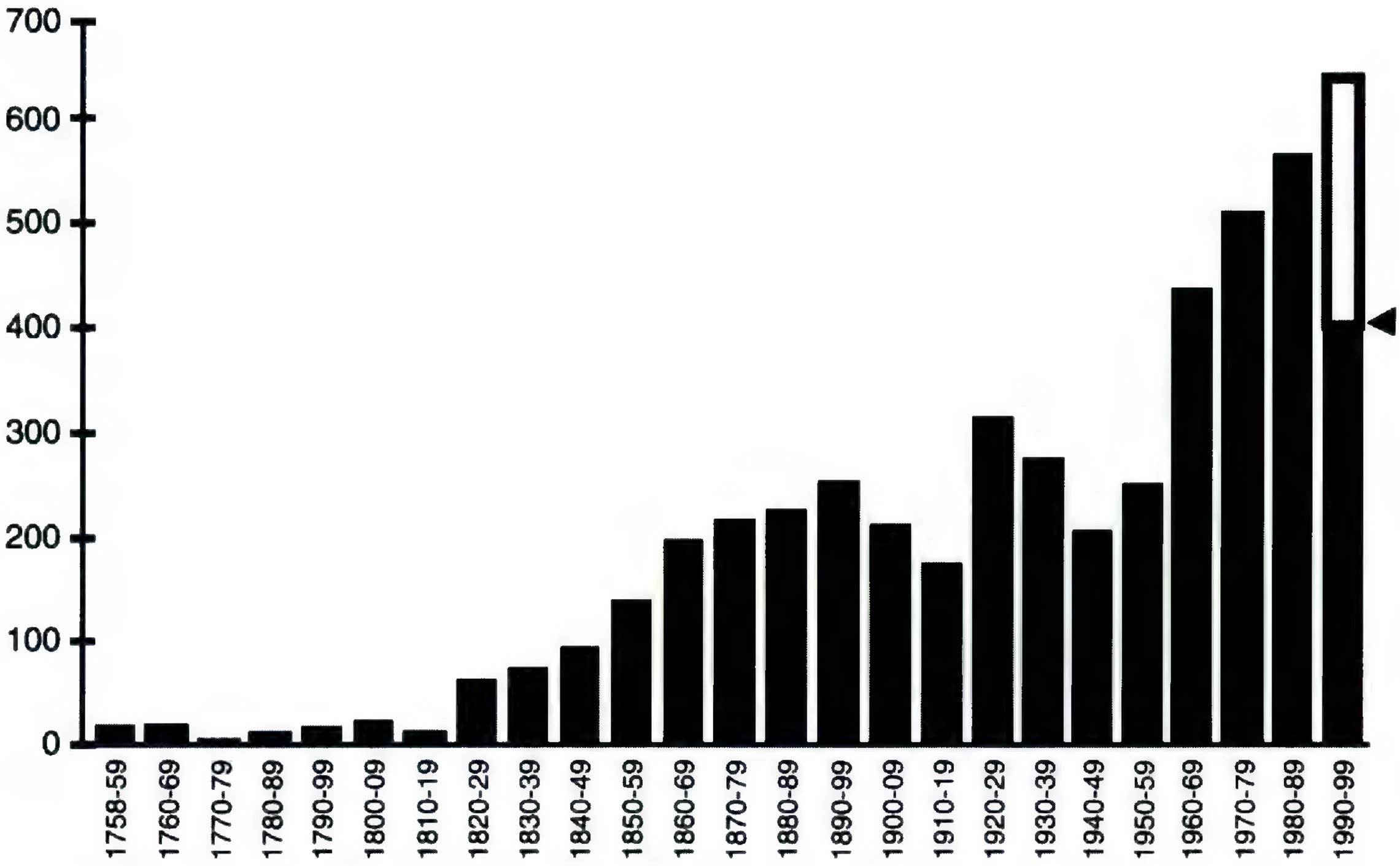

Figure 2. Amphibian species descriptions since 1758 (synonyms not included), clustered by decade, from Glaw and Köhler (1998). The arrow indicates the end of 1995; descriptions for the period 1996–1999 were extrapolated.

rious visitor a nearby specimen cabinet containing undescribed species.

By the criterion of the number of new species being described, we believe that the present compares favorably with any time since the mid-1700s. But how surprising are the new species that are being discovered in the late 20th century? Are we finding anything significantly different, or are we just filling gaps in an already well-known range of variation? John Horgan, author of *The End of Science* (published by Helix Books, in 1996), presents the latter view:

> "We are down to the details now. Every now and then something interesting will turn up. We will find a new species of lemur in Madagascar, or weird bacteria living in deep-sea vents. But at this point we are unlikely to discover something really surprising . . ." (J. Horgan, 1998).

This will sound absurd to those actively engaged in the discovery of new life forms, but such a "dotting the i's and crossing the t's" assessment of the situation is widespread both within the scientific community and the public at large.

To counter Horgan's naive but evidently persuasive claim, we need to consider criteria whereby a new species would qualify as "really surprising." Perhaps the primary criterion should be whether a new species fundamentally expands the way we think about the diversity and evolution of life. Unfortunately, this is much harder to quantify than counting the number of published descriptions of new species.

NEW PHYLA, ETC.

One possible indicator of the distinctiveness of newly discovered life forms, or perhaps of their informativeness with respect to our understanding of evolution, is the rate of description of new higher taxa. As Brusca (2000 this issue) shows, the description of new animal phyla does not appear to have diminished in recent years. Species are still being discovered that are considered by the relevant taxonomic communities to be as distinct as the familiar phyla are from one another. The major difference is that these new organisms are, on the whole, smaller, which means we are having to look more closely. Sometimes, as in the case of *Nanaloricus mysticus* of the recently described metazoan phylum Loracifera (Kristensen, 1983), this has entailed careful scrutiny of an obscure (though certainly widespread!) habitat—between sand grains on the ocean floor. In other cases new life forms have been found more or less right under our noses. *Symbion pandora*, a metazoan animal referred to the new Cycliophora, was described in 1995 from

a specimen collected on the mandible of a Norwegian lobster (Funch & Kristensen, 1995).

New "classes" of animals, sometimes with enormous significance for our understanding of major lineages, are also still being described at a regular pace. Examples include the Remipedia within Crustacea, discovered in a marine cave in the Bahamas (Yager, 1981), and the Concentricycloidea within echinoderms, found on waterlogged wood in deep waters off the coast of New Zealand (Baker et al., 1986).

In angiosperms there are excellent examples, mainly in the form of new families erected to accommodate extraordinary new species. For example, recall the excitement generated by *Ticodendron incognitum* Gómez-Laur. and L. D. Gómez (Ticodendraceae) from Costa Rica (Gómez-Laurito & Gómez P., 1989, 1991), and the subsequent realization that these trees occupy wet montane forests from southern Mexico to central Panama (Hammel & Burger, 1991). But the most spectacular case is probably *Lacandonia schismatica* E. Martínez and Ramos. This species of Triuridaceae-like monocot was described in 1989 in the new family Lacandoniaceae from material collected in the Lacandon region of Chiapas, Mexico (Martínez & Ramos, 1989). Astonishingly, its stamens are on the very inside of the flower (at the apex of the receptacle), surrounded by the carpels (Márquez-Guzmán et al., 1989; see Fig. 3a). This discovery, only a few years ago, came as a complete surprise—nothing about the other ca. 250,000 species of angiosperms predicted this brand new flower architecture. It also provides a unique opportunity to test the "ABC model" for genetic determination of flower organ identity (Coen & Meyerowitz, 1991; Vergara et al., 1999).

NOT PHYLA, ETC.

If the description of higher taxa were our only means of assessing the surprisingness of recent discoveries, we would miss many of the most exciting finds. The several examples below highlight radically new life forms (as different, we believe, as those discussed above), which were described only as new genera or as species within existing genera. These cases provide a dramatic reminder of the arbitrary nature and inadequacy of the Linnaean ranks—even in gauging the degree of disparity, which ranks are most often thought to represent.

In 1995, with very little fanfare, Dennis Desjardin and his colleagues described "an unusual psychrophilic aquatic agaric from Argentina" within the existing fungal genus *Gloiocephala* (Tricholomataceae). This remarkable new species, *G. aquatica,* develops its fruiting bodies and spores underwater, often beneath a layer of ice covering lakes in a zone between the Patagonian steppes and the Andes. As it appears to be the only known agaric to sporulate underwater, the term "unusual" is clearly an understatement—this is a brand-new way of being a mushroom. A similar discovery was also made in gastromycetes. In 1976, Escobar et al. described the genus *Limnoperdon* to accommodate the only known "aquatic" (actually, floating) puffball, which they found in marshes adjacent to the University of Washington in Seattle.

Amphibians also provide excellent examples of recently discovered lifestyles. In 1973, a new genus and species of leptodactylid frog, *Rheobatrachus silus,* was described on the basis of animals found in streams near Brisbane, Australia (Liem, 1973). Shortly thereafter, Corben et al. (1974) documented that *Rheobatrachus* exhibited a unique form of parental care. Brooding of the embryos and young takes place inside the stomach until the tadpoles or juvenile frogs are eventually (some eight weeks after ingestion) "propulsively ejected" through the mouth. This brooding mechanism is otherwise completely unknown in vertebrates. Furthermore, it requires special physiological mechanisms to turn off the secretion of gastric juices, which otherwise would kill the young. In 1983, Tyler et al. reported that prostaglandin secreted by the larvae inhibit acid production in the female's stomach "in a manner not seen elsewhere in the Animal Kingdom." Despite the potential medical significance of this remarkable phenomenon, further study will not be possible. *Rheobatrachus silus* appears to be extinct in the wild (Laurance et al., 1996).

In 1996, Nussbaum and Wilkinson described a new species and genus of caecilian (Amphibia: Gymnophiona), *Atretochoana eiselti.* As they documented (also see Wilkinson & Nussbaum, 1997), this is the largest lungless terapod, by far! It is 74–80 cm in length, while the next largest, a plethodontid salamander, is about ⅓ this size, at around 28 cm. The mouth is not connected to the nasal cavity in this animal, and its many other weird cranial features allow an exceptionally wide gape (see Fig. 4). These modifications are presumably associated with a novel feeding mechanism, but the natural history of these organisms is completely unknown. In fact, this extraordinary beast was "discovered" in a museum in Vienna and was known from only a single specimen collected from an unknown South American locality sometime in the last century. Now, another specimen has been located, this time in a jar in a Brazilian collection

Figure 3. Counterclockwise, from upper left. —a. Flower of *Lacandonia schismatica* (Lacandoniaceae, Triuridales), with three centrally positioned stamens surrounded and partially obscured by many papillose carpels. Photo courtesy of R. E. Alvarez-Buylla, E. Meyerowitz, and F. Vergara. —b. *Chrysaora achlyos* off the coast of northern Baja California, Mexico, in July 1989. The bell diameter is ca. 1 m and the combined oral arms, extending beyond the frame of photograph, are approximately 6 m long. Photo used with permission of J. Martin and Howard Hall Productions. —c. *Squamanita contortipes* parasitizing a host mushroom, probably *Galerina cerina*. Photo by S. A. Redhead.

(Wilkinson et al., 1998). Ironically, it, too, lacks a specific collection locality.

Other newly discovered species highlight how much we still have to learn. From Bacteria, consider *Prochlorococcus*, discovered only about a decade ago. Of great interest owing to the presence of both chlorophyll a and b (as in green plants), it is known now to be a major component of the phytoplankton in tropical and subtropical seas, and among the world's most important primary producers (see Fuhrman & Campbell, 1998). Moreover, the ability of *Prochlorococcus* to thrive in a wide range of light conditions seems to be explained by the existence of closely related but quite distinct species, living at different depths (Moore et al., 1998). Also of potentially huge ecological significance are new disease-causing bateria. For example, a newly discovered species of proteobacterium (related to

Figure 4. Lateral view of the head of the lungless caecilian *Atretochoana eiselti*. Its odd skull shape may reflect relaxed constraints on aspects of skull architecture involved with respiration and selection for increased gape size for prey capture. Photo by R. Nussbaum, from Wilkinson and Nussbaum (1997).

Sphingomonas) seems to be causing massive coral die-offs in Florida's reef communities (Richardson et al., 1998).

One of our favorite metazoan examples is the scyphozoan jellyfish *Chrysaora achlyos* (see Fig. 3b). This species was described by Joel Martin and colleagues in 1997, based on four specimens collected off the coast of southern California in the fall of 1989, and some photographs from northern Baja California (Martin et al., 1997). One of several species in this genus, *C. achlyos* is remarkable by virtue of being enormous. In fact, with a bell measuring over 1 m in diameter, this is the largest invertebrate described in this century, rivaled in size only by the giant squid and an arctic jellyfish described in the 1800s (J. Martin, pers. comm.). Although thousands washed ashore in 1989, it has not been seen since.

From vertebrates comes the story of the salamander *Eurycea sosorum*. This species was described in 1993 from specimens collected in the Barton Springs Aquifer, within the city of Austin, Texas (Chippendale et al., 1993). Known only from a single popular swimming spot, and therefore in grave danger of extinction, this species continues to attract considerable attention in Austin. Subsequent exploration of nearby springs, some located within Austin housing developments, has turned up dozens of new species (D. Hillis, pers. comm.). Description of these species, from a relatively small area well endowed with herpetologists, will increase the number of described salamanders of the world by about seven percent!

Just as numerous new amphibians are coming to light, so, too, are novel pathogens that prey upon them (Hanken, 1999). A new genus and species of chytrid fungus, *Batrachochytrium dendrobatidis*, described very recently (Longcore et al., 1999), appears to be a major proximate cause of mass mortality events in frogs around the world (Berger et al., 1998; Morell, 1999). Being the only chytrid known to prey on vertebrates, this new species also greatly expands our understanding of a major and ubiquitous fungal group (Longcore et al., 1999; Pessier et al., 1999).

Seed plants also provide fine examples. *Wollemia nobilis* W. G. Jones, K. D. Hill & J. M. Allen, the sister group of *Agathis* (Gilmore & Hill, 1997) or

possibly all other Araucariaceae (Setoguchi et al., 1998), and closely resembling a fossil known from the Cretaceous through the Oligocene, was discovered in 1994 growing in the Wollemi National Park, ca. 200 km northwest of Sydney, Australia (Jones et al., 1995; Briggs, 2000 this issue). How could a large tree, with such distinctive architecture, growing near the largest city in Australia, have escaped detection until 1994?

At about the same time, Andrew Douglas and colleagues were realizing that collections of an odd proteaceous tree made in the 1960s and 1980s from northeastern Queensland represented a new species, *Eidothea zoexylocarya* A. W. Douglas & B. Hyland. These 40-m-tall plants, seemingly endemic to rainforests in the Bloomfield River watershed, are the sole representatives of an apparently basal lineage of Proteaceae, the Eidotheoideae (Douglas, 1995; Douglas & Hyland, 1995). Its fruits are nearly identical to 60-million-year-old fossils of *Xylocaryon lockii* F. Muell. (Holden, 1995).

Neviusia cliftonii Shevock, Ertter & D. Taylor (Rosaceae, Kerrieae) provides a similar example (Ertter, 2000 this issue). This species was described in 1992 from material collected south of Mount Shasta in northern California (Shevock et al., 1992). Although it is a rather conspicuous and locally abundant shrub and grows at several localities in an area where the vegetation had previously been mapped, it seems never to have been collected before 1992. As the authors speculated, the abundance of poison-oak in the vicinity may have been enough to discourage thorough exploration. Judging by its resemblance to an Eocene fossil from British Columbia, *N. cliftonii* is probably a relict in this area (Shevock et al., 1992). Its closest living relative, *Neviusia alabamensis* A. Gray, is known from only a few localities in Alabama and Arkansas.

Attention to Phylogeny

The ongoing age of discovery differs importantly from earlier episodes by virtue of the attention being paid to phylogeny. The phylogenetic notion of relationship was nonexistent before the mid-1800s, and not until recently have new methods and sources of evidence allowed rigorous and (we trust) increasingly accurate phylogenetic inferences. Although biodiversity is still commonly equated with the enumeration of species (and genetic diversity within species), the idea has now expanded. Biodiversity is not just about species, at the tips of the tree, but about the whole tree of life. Thus, finding any branch of the tree, external or internal, constitutes a genuine act of discovery. Viewed in this light, it is counterproductive to draw too strong a boundary between discovering species and discovering clades. These activities are pieces of the same underlying project, namely, understanding the entire tree of life.

Addition of this phylogenetic dimension makes the ongoing age of discovery far richer, and certainly far more powerful in connection with practical problems such as, for example, the choice and design of nature reserves and the search for useful natural products. In general, knowledge of phylogenetic relationships brings with it the power of prediction. Phylogenetic research should therefore be viewed as a welcome addition to, rather than a competitor with, the study of species diversity.

Consequently, it is of great interest to consider, as we have for species, both the rate of discovery of new clades and the surprisingness of these new discoveries. Unfortunately, we know of no straightforward way to quantify these things. There is little baseline information regarding the rate of discovery, and databases such as TreeBASE (http://phylogeny.harvard.edu/treebase), which could eventually be a ready source of such information, have grown too unevenly to provide an accurate measure of clade discovery. Nevertheless, the rate appears to be growing exponentially, and shows no signs of tapering off (Sanderson et al., 1993). At least this is the case if the number of clades being discovered grows in proportion to the rapidly increasing number of phylogenetic analyses being published, which we have no reason to doubt.

In theory, one might approximate the rate of discovery by tallying new names applied to clades, but at present this information is not being databased consistently enough. However, it is important to appreciate that even if this were now possible, the number of clades actually being named is far less than the number of clades being discovered, or even the number of clades identified with great confidence. Reluctance to formally name clades stems in part from the traditional Linnaean nomenclatural system's emphasis on the assignment of taxonomic rank, and the potential consequences of such assignments on the application of names at other levels (Baum et al., 1998; Hibbett & Donoghue, 1998). Related to this is the realization that there are not enough familiar ranks in the Linnaean system to do justice to the many nested clades being discovered even in average-sized phylogenetic analyses. In practice, the current nomenclatural codes (e.g., Greuter et al., 1994) encourage formally naming clades only after relationships throughout the group in question have been rather well estab-

lished, as opposed to encouraging the naming of well-supported new clades as they are discovered, usually one or a few at a time (Hibbett & Donoghue, 1998).

The surprisingness of recent discoveries is also hard to judge because prior views on relatedness have often not been expressed unambiguously enough. Nevertheless, it is clear that many entirely unexpected discoveries have been made. We now appreciate, for example, that "prokaryotes" are not a single branch, but two (or more) major branches, with lineages of Archaea probably more closely related to eukaryotes than they are to Bacteria (e.g., Pace, 1997). Likewise, we can say with growing confidence that animals and fungi (Baldauf & Palmer, 1993; Wainright et al., 1993), and possibly also slime molds (Baldauf & Doolittle, 1997), are more closely related to one another than they are to plants or any other eukaryotes.

While these are obviously major advances in our understanding of life, such discoveries may not seem terribly surprising since relationships of the major lines of life have always seemed obscure. What about within metazoan animals and green plants, where phylogenetic theories have been better developed—have any really surprising results emerged from recent studies?

The answer is an unambiguous yes! Relationships among major metazoan lineages have come into much sharper focus, and a number of traditional views have been overturned. For example, whereas it has long been assumed that annelids are more closely related to arthropods than they are to molluscs (as evidenced by segmentation and the existence of seemingly intermediate onycophorans), much recent evidence points instead to the existence of a euthrocozoan or lophotrochozoan clade, which includes annelids and molluscs, to the exclusion of arthropods (Eernisse et al., 1992; Halanych et al., 1995).

More astonishing, perhaps, is the possibility of an ecdysozoan clade, which includes all of the molting animals, and therefore unites arthropods with such groups as onycophorans, tardigrades, and even nematodes (Aguinaldo et al., 1997; Giribet & Ribera, 1998; Knoll & Carroll, 1999). And within arthropods (Fortey & Thomas, 1997; Brusca, 2000), there is mounting evidence for the union of crustacea and insects and even the paraphyly of crustacea with respect to insects (and possibly the other major arthropod groups as well). Among other things, this implies convergent evolution of adaptations to life on land (tracheal systems, Malpighian tubules, etc.) in hexapods and myriopods (traditionally the Atelocerata).

Even within mammals, where one supposes that many relationships have long since been established with certainty, there have been some major surprises in the last few years (de Jong, 1998). Our favorite case concerns the position of elephant shrews and golden moles. Once considered insectivores, these are now seen to be united with aardvarks, hyraxes, sea cows, and elephants, which implies extraordinary morphological radiation within a basically African clade (Springer et al., 1997; Stanhope et al., 1998).

Within green plants, molecular phylogenetic studies have tended to confirm earlier suggestions based on morphology (Donoghue, 1994), but there are also some very surprising results, of which we mention only a few (see Doyle, 1998, for others). The whisk-ferns, Psilotaceae, now appear to be related to Ophioglossales (e.g., Hasebe et al., 1995; Manhart, 1995; Wolf, 1997), as opposed to being remnant rhyniophytes or related to some group of leptosporangiate ferns, as suggested previously. Within ferns, the aquatic and heterosporous Salviniaceae and Marsileaceae, long viewed as unrelated, now appear to be directly linked (e.g., Pryer et al., 1995; Wolf et al., 1999), a connection also supported by fossil evidence (Rothwell & Stockey, 1994). Within angiosperms, the tricolpate or eudicot clade, recognized first in morphological analyses (Donoghue & Doyle, 1989; Doyle & Hotton, 1991), has been rather consistently upheld in molecular studies (e.g., Chase et al., 1993; Rice et al., 1997; Soltis et al., 1998; and even the highly unresolved parsimony jackknife analysis of Källersjö et al., 1998). This represents a real departure from the traditional view that caryophylids, hamamelids, and other major lineages were derived independently from magnoliids (e.g., Cronquist, 1988). Other surprises include *Drosera* and *Nepenthes* being related to caryophillids (Albert et al., 1992), a clade including almost all plants with mustard oils (Rodman et al., 1996), and a connection of woody Hamamelidaceae and *Cercidophyllum* with herbaceous Saxifragaceae and relatives (Soltis & Soltis, 1997).

Some of the most interesting early results, such as the basal position of *Ceratophyllum* within angiosperms in *rbc*L analyses (e.g., Chase et al., 1993), have not been consistently recovered as additional data have been brought to bear. But other seemingly weird results have been upheld. Of these, we are most excited about the possibility that Proteaceae, Platanaceae, and *Nelumbo* are positioned near the base of eudicots, and may form a clade (Chase et al., 1993; Rice et al., 1997; Soltis et al., 1997; Hoot et al., 1999). Based on outward

appearances, one would never guess that these plants are closely related, and a direct relationship of the three had never been suggested. On closer consideration, this arrangement is not illogical from a morphological standpoint, though it has proven quite difficult to identify any morphological synapomorphies (Hoot et al., 1999; A. Douglas, pers. comm.).

Although many phylogenetic problems have been convincingly resolved in recent years, the relationships of many groups remain unclear. In most cases, this reflects nothing more than limited attention, and we can expect straightforward solutions. Yet, in some other cases, relationships remain unresolved despite considerable attention. For example, it is fair to say that relationships among major mammal lineages are still up in the air (de Jong, 1998). Likewise, relationships among major seed plant groups have still not been satisfactorily resolved (Doyle, 1998; Hansen et al., 1999; Winter et al., 1999). Within flowering plants, perhaps the most remarkable confusion surrounds Rafflesiales. While the position of most of the other parasitic groups has fallen into place, the relationships of Rafflesiales remain highly uncertain, despite great efforts on the part of Nickrent and colleagues (Nickrent et al., 1998). In fact, at this stage it is still unclear whether Rafflesiales are eudicots, or are instead more closely related to one or another monosulcate group (as Hydnoraceae now appear to be related to Aristolochiales/Piperales). A remarkably accelerated rate of evolution of the nuclear ribosomal genes of these plants may be creating artifacts in phylogenetic analyses (e.g., placement of Rafflesiales at the very base of angiosperms; Nickrent & Starr, 1994; Nickrent & Duff, 1996).

What can we expect in the near future? First, and most obviously, the discovery of clades is still in its infancy, and many more breakthroughs are on the horizon. We expect that even the most recalcitrant problems, including those just highlighted, will eventually yield to more data and better methods of analysis.

Second, we trust that more attention will be paid to the naming of newly discovered clades, so as to facilitate communication about exciting results as rapidly, unambiguously, and with as little nomenclatural disruption as possible. For this purpose we believe that a new phylogenetically oriented code of nomenclature is needed (see above). Fortunately, such a phylogenetic code is being developed as a result of a workshop held at the Harvard Herbaria in August 1998. This will soon be available via the internet for evaluation and, subsequently, for formal use. In this system there are no categorical ranks, and names are applied to clades by means of phylogenetic definitions (e.g., a "node-based" definition, such as "the least inclusive clade that contains species X and species Y"; de Queiroz & Gauthier, 1992, 1994). This, we believe, represents a major and very positive shift away from arbitrary decisions about rank and the need to appeal to authority (e.g., Cronquist, 1988; APG, 1998) concerning the circumscription of taxa. One would immediately know, for example, whether or not "Bombacoideae" is included in "Malvaceae," if both names were given phylogenetic definitions and if a particular phylogenetic hypothesis were accepted (Baum et al., 1998; Alverson et al., 1999); without phylogenetic definitions, botanical authorities could and probably would disagree about this, even if they accepted the same underlying phylogeny for their nomenclatural decisions.

Finally, as emphasis shifts toward phylogenetic knowledge, the need to database such information will become increasingly obvious, both to track progress and to render phylogenetic discoveries accessible to a very wide variety of potential users. Several complementary efforts have been initiated to keep track of and synthesize this information, including TreeBASE (see above) and the Tree of Life project (http://phylogeny.arizona.edu/tree/phylogeny.html). However, owing to the exponential increase in phylogenetic research, and limited support for these projects, they have not been able to keep pace and are therefore still inadequate for many purposes. We hope that this situation will improve as journals move toward electronic submission of phylogenetic data sets as a co-requisite of publication (see, e.g., *Mycologia*), perhaps also linked with the registration of clade names. In any case, this is a problem that the systematics community will need to address in order to capitalize on the major investments being made in phylogenetic research.

Attention to Geography

The present age of discovery also differs in the attention being given to geographic ranges. Collection localities were barely a consideration until quite recently. Recall, for example, Darwin's difficulties in reconstructing (after the fact) the islands from which his Galápagos finches were actually collected (Sulloway, 1982). In contrast, we now place great value on detailed, accurate specimen labels and on databasing geographic information. Many of the most important questions about the organization of biodiversity depend heavily on such information, such as the distribution and relative "hotness" of

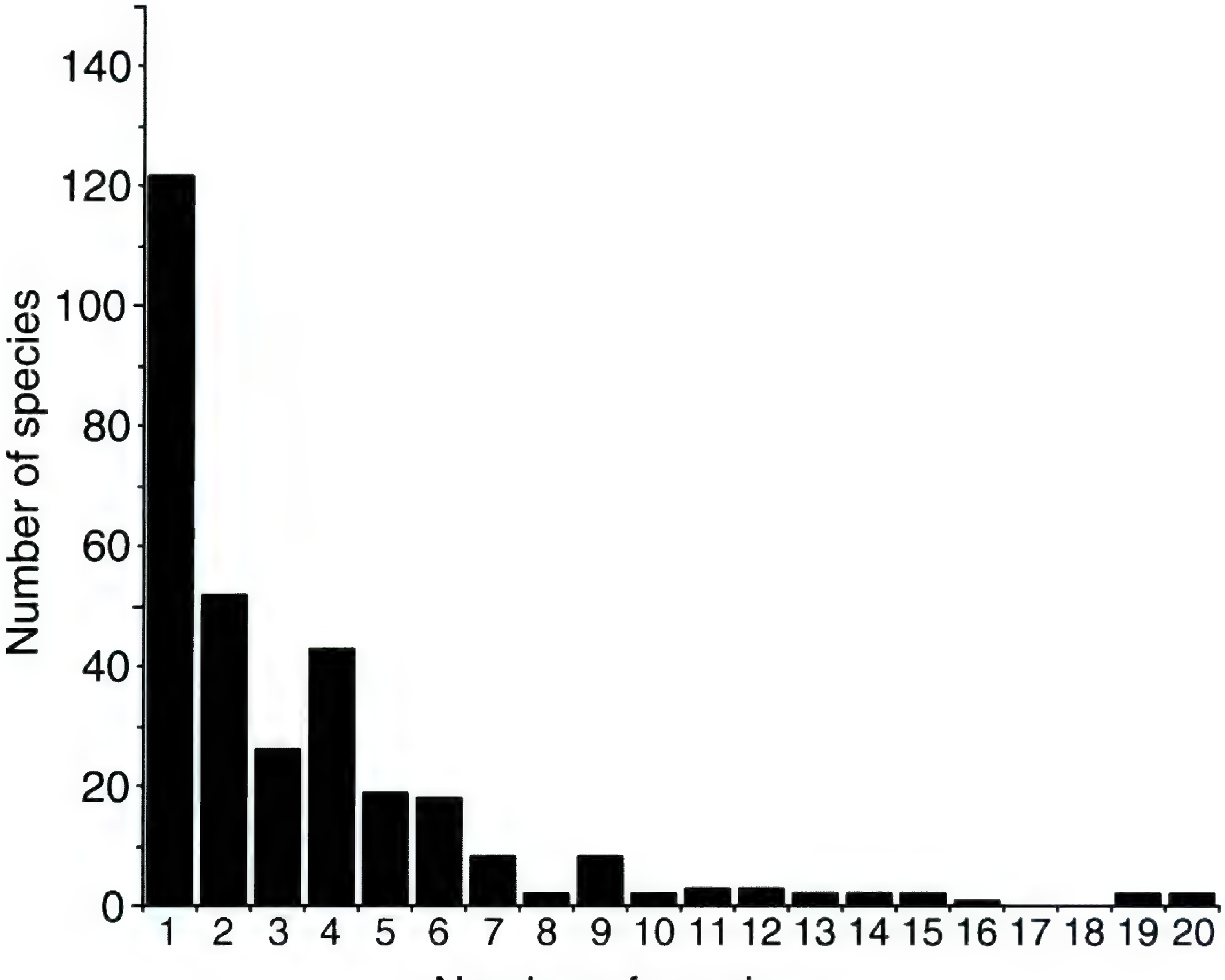

Figure 5. Number of specimens for species of woody tropical plants. The data represented in this graph were obtained by Madriñán-Restrepo (1996) from recent monographs of neotropical plants: 317 species were scored in 15 genera from 8 families: *Rollinia* (Annonaceae), *Licania* (Chrysobalanceae), *Agarista, Gaultheria* (Ericaceae), *Aiouea, Aniba, Mezilaurus, Nectandra, Pleurothyrium, Rhodostmonodaphne* (Lauraceae), *Eschweilera, Gustavia* (Lecythidaceae), *Guarea* (Meliaceae), *Pouteria* (Sapotaceae), and *Arytera* (Sapindaceae).

diversity hotspots (Reid, 1998), or the existence of refugia in Amazonia (Nelson et al., 1990). Moreover, many critical practical applications of taxonomic knowledge, such as estimating the impact of human disturbance on species loss (e.g., Pimm et al., 1995), are impossible without reasonable knowledge of geographic distributions.

Where, then, do we stand in our knowledge of geographic ranges? This question is difficult to answer, since baseline data have not been compiled in an appropriate fashion. However, there are several reasons to believe we are still very far from having an accurate picture. One clear indication is the fact that many species are known from one or a few museum collections, and these are often old and lack sufficiently detailed locality data. The lungless caecilian, *Atretochoana eiselti,* which we highlighted above, provides a striking example: neither of the two collections of this remarkable species provides locality data more specific than "South America."

Data collected by Madriñán-Restrepo (1996) from monographic treatments of 15 tropical plant genera (Fig. 5) illustrate a situation that is undoubtedly common. Of the 317 species he considered, 122 (38.5%) were known from just one specimen, 52 (16.4%) from two specimens, and 26 (8.2%) from three specimens. That is, more than half were represented only by one or two specimens, and almost two-thirds by three or fewer. Few of the species he scored were known from more than 10 specimens, and none from more than 20. In such cases there are simply insufficient data to draw reasonable inferences about geographic range.

It is possible, of course, that species known from one or two specimens or localities are truly very

restricted in occurrence. However, in most cases subsequent fieldwork indicates otherwise. Detailed data on this point have not been assembled, but we know that in most groups of organisms every field trip yields range extensions, sometimes major ones. A good example is provided by recent fieldwork in a small corner of Qinghai, China's fourth largest province, with an area of around 720,000 km^2 (about 6% larger than the state of Texas). Before an expedition in 1995, only four bryophyte species were reported in the literature for all of Qinghai. Three weeks of moss collecting by Benito Tan in Yushu Prefecture alone yielded 57 genera and 109 species (Tan & Yu, 1997). Two of these species were new to science, three were previously unknown in China, and ten collections represented major range extensions within China.

Even in groups of organisms and in places that have been better collected, key elements have often been overlooked. In 1998, our colleague in the Harvard Herbaria, Gustavo Romero, collected *Erisma japura* Spruce ex Warm. (Vochysiaceae) from the Isthmus of Pimichin (between the Orinoco and Amazon basins) in Venezuela. The first collection of this species from Venezuela had been made only the year before, from the same area; previously, it was known from a few collections along the Rio Negro in Colombia and from Brazil. What makes this remarkable is that Alexander von Humbolt and Aimé Bonpland, Richard Spruce, Alfred Wallace, Llewelyn Williams, Julian Steyermark, Bassett Maguire, and others, all worked here, but none of them collected *Erisma japura*, despite the fact that it appears to be the dominant tree in the non-flooded forests of the area. Individuals range to 35 m in height and over 1 m in diameter, and have conspicuous winged fruits that are used as a source of starch by local people (G. Romero, pers. comm.).

Two other discoveries, also of large neotropical trees, emphasize that our knowledge of the distribution of many clades is still rudimentary. *Rupti-liocarpon caracolito* Hammel & N. Zamora was described in 1993, primarily on the basis of collections from Costa Rica's Osa Peninsula (Hammel & Zamora, 1993). Its closest relative appears to be *Lepidobotrys* (Lepidobotryaceae) of the Gabon-Cameroon region of Africa. In a similar case, *Pseudomonotes tropenbosii* A. C. Londoño, E. Alvarez & Forero was described in 1995 from the Colombian Amazon (Londoño et al., 1995). This is only the second New World species of the otherwise Old World Dipterocarpaceae, which dominate the tropical forests of Asia. The other neotropical species, *Pakaraimaea dipterocarpacea* Maguire & Ashton, was discovered less than 20 years earlier (Maguire & Ashton, 1977).

Examples like these are surely common, but are they really very surprising? After all, one could argue that these are not organisms about which we thought we had good knowledge to begin with. Have we learned anything really new about the distributions of organisms that we thought we knew well, organisms of great enough interest that conscious efforts had been made to find them and establish their ranges?

Again, the answer is a definitive yes! The most striking recent case concerns the coelacanth, *Latimeria chalumnae*. Thought to have been extinct since the Upper Cretaceous, and of great evolutionary interest from the standpoint of the origin of tetrapods, a specimen was caught off the coast of South Africa in 1938. After an intensive search, a second specimen was found in 1952 near the Comoro Islands, northwest of Madagascar (Thomson, 1989). Since then, more than 100 additional specimens have been taken of the now endangered fish in the vicinity of the Comoro Archipelago, but nowhere else. It therefore came as a great shock when, on the 30th of July, 1998, a coelacanth was caught in a gill-net off the coast of north Sulawesi, Indonesia, almost 10,000 km away from the Comoros (Erdmann et al., 1998; see note p. 126). Other populations may exist between the two sites, but in any case this discovery reminds us how little we know about the distributions of even large and well-publicized marine organisms.

Similar lessons are provided by the rediscovery of species thought to have become extinct in recent time. Botanists will be aware, for example, of the rediscovery of *Takhtajania perrieri* (Capuron) Baranova & J-F. Leroy in northeastern Madagascar, about 150 km east of the spot where the original and only collection was made 85 years earlier (Schatz et al., 1998). Being of great interest from the standpoint of angiosperm evolution (as the only modern African-Madagascar representative of the vesselless Winteraceae), botanists from the Missouri Botanical Garden and their Malagasy colleagues had tried in vain since 1974 to relocate *Takhtajania*. A Malagasy collector stumbled upon a population of over 250 adults while doing inventory work in 1994, and study material has been made available to botanists around the world. Molecular phylogenetic analyses now suggest that *Takhtajania* is the sister group of the rest of the Winteraceae (E. Zimmer, pers. comm.).

Even better known rediscoveries involve vertebrates, especially mammals. Again from Madagascar comes the story of the rediscovery of the greater

bamboo lemur, *Hapalemur simus* (Wright, 1988), which was thought to have become extinct in 1900. A living animal was purchased in a market in 1964, but soon escaped from captivity; not until 1972 was a small population found in the wild. In 1986, two expeditions, one led by Patricia Wright from the United States and the other by Bernhard Meier of Germany, were successful in locating it again. This set the stage for detailed studies of behavior and ecology, which led in turn to the realization that there were actually two separate species involved. The new species, which they called the golden bamboo lemur, *H. aureus* (Meier et al., 1987), apparently specializes on new shoots of the bamboo plant, exceptionally rich in the poison cyanide.

Two fungal examples further emphasize the potentially great importance of such discoveries. The mushroom *Squamanita contortipes*, first described in 1957 (in the genus *Cystoderma*), was rediscovered in 1992 during the course of systematically sampling the macrofungi of old-growth spruce-hemlock forests on the Olympic Peninsula of Washington (Redhead et al., 1994). As shown in Figure 3c, *S. contortipes* fruit bodies were found growing from the cap of another agaric, a species of *Galerina*. This observation provided the crucial clue to the interpretation of the lifestyle and morphology of an entire group of fungi. *Squaminita* species were initially described as having "protocarpic tubers," sometimes mysteriously resembling the tissues of distantly related mushrooms. Owing to the Olympic Peninsula specimens, these structures are now understood to be deformed hosts, and *Squaminita* is now recognized to be only the second agaric genus in which all species are obligate parasites of other basidiomycetes.

In 1994, a group of Cornell students taking a field mycology course in upstate New York encountered unusual fruiting bodies growing out of the backs of dead beetle larvae. These turned out to be the ascomycete *Cordyceps subsessilis*. Propagation of this material in the laboratory revealed it to be the formerly unknown sexual stage (teleomorph) of the mold *Tolypocladium inflatum*, which produces the immunosuppresive compound cyclosporin, a billion-dollar drug used in preventing the rejection of transplanted organs (Hodge et al., 1996). This knowledge makes possible, for the first time, the search for other pharmaceutically important chemicals (possibly of use in treatment of autoimmune diseases) in related *Cordyceps* species.

What developments do we anticipate with respect to geographic ranges? Most obviously, it will be critical to continue databasing geographic information associated with museum specimens and making this accessible to potential users. This will give a much better picture of the distributions of species and clades, but will still be insufficient for many purposes, both for lack of collections, and because, as several of our examples illustrate, a set of collection localities is not the same as a range, and may even be a poor approximation of the entire distribution. A critical step will, therefore, be the development and application of models for estimating real geographic ranges from sets of collection sites. Fortunately, great strides have been made in this area with the development of Geographic Information Systems (GIS) and models such as those underlying BIOCLIM/BIOMAP, for example (see Austin, 1998; Scott & Jennings, 1998).

Another exciting prospect entails improved connections between geographic and phylogenetic information. This would not only revolutionize the study of historical biogeography, but would also provide a genuinely new perspective on the distribution of diversity as this relates to conservation and management issues (e.g., Vane Wright et al., 1991). Already there are concrete examples, involving a range of organisms and spatial scales. For instance, phylogenetic analyses point to Papua New Guinea as a hotspot of genetic diversity in shiitake mushrooms, and therefore a conservation priority (Hibbett & Donoghue, 1996). Analyses in Asteraceae and curculionid beetles highlight the significance of southern temperate regions in relation to global biodiversity (Morrone et al., 1996). Similarly, in the north temperate zone the Pacific Northwest harbors a variety of species of tremendous phylogenetic significance (D. Wake, pers. comm.), such as *Ascaphus truei*, the sister group of all modern frogs (Ford & Cannatella, 1993). Ultimately, we envision a concatenation of databased geographic information (especially predicted ranges along the lines of BIOCLIM/BIOMAP) with databased phylogenetic information (along the lines of TreeBASE). Among many other things, this would enable the wide-scale application of phylogenetic measures of diversity (e.g., Williams et al., 1991; Faith, 1992).

Concluding Thoughts

To systematists, it is probably already clear that we live in an age of discovery and that this one is richer and more profound by virtue of the attention being paid to both phylogeny and geography. Yet, this is not at all obvious to much of the scientific community or to the general public, who tend to regard the discovery of diversity as a tedious filling-

in of minor gaps in our already comprehensive knowledge. Whether or not we have failed, ourselves, to fully appreciate the number and significance of recent discoveries is debatable. What is not debatable is that we have failed to effectively deliver news of these stunning discoveries to those outside of our community. As we have suggested, there are a variety of reasons for this, including our limited ability to compile information on the number of newly discovered species and clades, or to convey the surprisingness of new discoveries. We also are limited by a system of nomenclature that tends to discourage rather than facilitate a full elaboration of the tree of life. These issues need to be confronted directly, and soon.

In the meantime, there are several avenues along which we can advance public awareness and appreciation of biodiversity. Perhaps most obviously, we can highlight exactly how it is that understanding and protecting biodiversity is in our own best interest, even economically. It is hard to imagine a more compelling statement of this connection than the "Teaming with Life" document prepared recently by the Biodiversity and Ecosystems Panel of the President's Committee of Advisors on Science and Technology (PCAST; Raven et al., 1998). Big and bold new biodiversity discovery projects are another mechanism to capture the public's imagination. For example, we see enormous educational potential in the All Taxa Biodiversity Inventory (ATBI) being undertaken by the Great Smoky Mountains National Park (www.discoverlife.org). Several of the projects to be sponsored as part of the DIVERSITAS International Biodiversity Observation Year (IBOY) in 2001 (www.icsu.org/diversitas) have similar potential: for example, retracing key voyages of past ages of discovery (by Darwin, Banks, von Humboldt, and Wallace), or assembling an enormous tree of life and somehow physically displaying its grandeur to the public, or launching major international expeditions to assess diversity in little known regions or in extreme environments.

New discoveries of the sorts we have highlighted have enormous potential to inspire commitment to the project of discovering the diversity of life. However, to fully capitalize on this potential, we will need to pay more attention to specific mechanisms for awakening wonder. Is it possible to induce the sense of awe, amusement, and even befuddlement that remarkable new organisms inspired during the last great age of discovery? What is it that inspires wonder in six-year-old children visiting a natural history museum for the first time? Along these lines we are encouraged by several recent developments. In a fine analysis of the aesthetics of biological diversity, Kiester (1997) drew attention to the existence of a wide range of biodiversity experiences, contrasting those centered on the immediate, tangible beauty (or bizzareness) of individual organisms with those invoking a sense of the vastness or power of nature (what Immanuel Kant called "sublime" experiences). If we pay closer attention to such distinctions, the range of experiences triggered by biodiversity discoveries could surely be expanded, and their impact could be far greater on the public's perception of nature and the discovery process itself.

In view of this, we should renew our attention to how new discoveries are brought forward in natural history museums and elsewhere. We are delighted by recent experimentation with museum exhibits, especially those inspired by the "wonder cabinets" of the past (Hutchinson, 1965; Weschler, 1995). These collections of rarities—natural objects often commingled with works of art and assembled in seemingly haphazard fashion—help put the observer slightly, but very constructively, off balance. Perhaps the premier modern experiment of this type is David Wilson's Museum of Jurassic Technology in Los Angeles, California (Weschler, 1995). By playfully straddling the dynamic boundary between reality and fiction, Wilson seems to have hit upon an unusually successful way of stimulating doubt and wonder. Today's natural history museums, which sometimes convey an unwarranted certainty or finality about our knowledge of the world, would benefit from careful scrutiny of the Wilson model. There is great power, we believe, in the mystery and strangeness of life, and in deeply appreciating how little we still know about it. The torrent of biological novelties now coming to light may provide the very best way to tap that power.

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Note added in proof. The coelacanth specimens from Indonesia have been named as a new species, *Latimeria menadoensis*, a conclusion seemingly well supported by molecular data (see M. T. Holder, M. V. Erdmann, T. P. Wilcox, R. L. Caldwell & D. M. Hillis. 1999. Proc. Natl. Acad. Sci. U.S.A. 96: 12616–12620; and L. Pouyaud, S. Wirjoatmodjo, I. Rachmatika, A. Tjakrawidjaja, R. Hadiaty & W. Hadie. 1999. Compt. Rend. Acad. Sci. Paris, sér. 3, 322: 261–267.

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REPRODUCTIVE BIOLOGY OF *ERYTHRINA CRISTA-GALLI* (FABACEAE)[1]

Leonardo Galetto,[2] *Gabriel Bernardello,*[2] *Irene C. Isele,*[3] *José Vesprini,*[4] *Gabriela Speroni,*[5] *and Alfredo Berduc*[3]

Abstract

Flowering phenology, floral morphology, nectar features (chemical composition, secretion pattern, standing crop, removal effects), breeding system, and flower visitors were analyzed in seven populations of *Erythrina crista-galli* from Argentina and Uruguay. Its flowering phenology corresponds to the sub-annual frequency class of Newstrom et al., with a major burst in November. Flowers lasted for 3 to 4 days and produced abundant hexose-dominant nectar with its chemical composition and concentration (ca. 22%) constant across all flowering stages. Most of the total nectar was secreted by buds. When the flowers first opened, most (> 50%) of the total nectar was available to pollinators. As flowers faded, a resorption period began. The overall sugar production was not affected by nectar removal. Hand crosses showed that this species is self-compatible. Crossed fruits showed significant differences from hand-selfed ones (autogamy and geitonogamy). Xenogamous fruits and seeds showed the highest values for many traits (fruit mass, total seeds per fruit mass, mean seed mass, and seed germination percentage). Approximately 6% of the flowers set seeds in natural populations. Hymenopterans (carpenter bees and honeybees) and hummingbirds (four species) assiduously visited the trees in all the areas sampled and can be assumed pollinators. Almost 93% of recorded flowers were visited by bees, with the rest visited by hummingbirds. Phylogenetically, this species was included in the basal clade for the genus and characterized as passerine/hummingbird pollinated. However, we found that not only birds but bees functioned as major pollinators. This observation may indicate that this basal clade may represent an intermediate step from entomophily (typical of tribe Phaseoleae) to ornithophily (typical of *Erythrina*).

Key words: breeding system, *Erythrina,* Fabaceae, flower visitors, nectar features, nectary.

Ecological and evolutionary success of the legumes has been strongly related to highly successful biotic pollination mechanisms, utilizing mostly bees, birds, and bats (Kalin Arroyo, 1981; Schrire, 1989). Although much is already known about the pollination biology of Fabaceae, as indicated by

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Kalin Arroyo (1981) and Schrire (1989), their data are too narrowly focused to enable a detailed synthesis. Thus, we emphasized a thorough analysis of several aspects of the pollination biology in several populations of one legume species.

Erythrina crista-galli L. was chosen because this genus is well known from a number of studies (see references following). *Erythrina* comprises about 112 species, mostly arborescent and pantropical, but also extending into warm-temperate areas, such as Argentina (Krukoff & Barneby, 1974). It is assigned to the large, economically significant papilionoid tribe Phaseoleae. Recent cpDNA restriction site analyses of this tribe suggest that *Erythrina* is highly derived (Doyle & Doyle, 1993; Bruneau et al., 1995). The diversity of floral morphology may be associated with differences in pollination systems (Bruneau, 1997). Among the primarily entomophilous Phaseoleae, *Erythrina* is atypical in being ornithophilous (Neill, 1987; Bruneau, 1997), providing good examples of adaptation to different types of birds (Proctor et al., 1996). It shares with other bird-pollinated groups the red or orange, odorless flowers with copious nectar, and diurnal anthesis (Proctor et al., 1996; Bruneau, 1997). Most American species are pollinated by hummingbirds (Neill, 1987; Bruneau, 1997), although some are passerine pollinated. Both guilds of birds tend to be species specific (cf. Bruneau, 1997).

Phylogenetic hypotheses considering morphological and cpDNA restriction site characters suggest that shifts from passerine bird to hummingbird pollination have occurred several times in the genus (Bruneau, 1997). *Erythrina crista-galli* is included in a paraphyletic assemblage of South American species that are basal in the genus (Bruneau & Doyle, 1993; Bruneau, 1996). Thus, a detailed study of the reproductive biology of this species may provide clues to the evolution of the pollination biology within the genus.

Erythina crista-galli is native to southern Brazil, Bolivia, Paraguay, Uruguay, and Argentina as far as the Río de La Plata, having the southernmost distribution for the genus in the Americas. The species consists of trees to 10 m tall, with non-tubular, large, red flowers of ornamental, economic, and medicinal interest (Burkart, 1987). It is cultivated throughout the world in frost-free climates (Krukoff & Barneby, 1974) and is the national flower of Argentina and Uruguay.

Scattered reports are found on its reproductive biology, mainly reporting floral visitors, breeding system, or nectar chemical composition (Knuth, 1906; Schrottky, 1908; Werth, 1915; Pickens, 1931; Ali, 1932; Fryxell, 1957; Faegri & van der Pijl, 1971; Raven, 1974; Toledo, 1974; Parodi, 1978; Toledo & Hernández, 1979; Kalin Arroyo, 1981; I. Baker & H. G. Baker, 1979; H. G. Baker & I. Baker, 1983, 1990; Neill, 1987, 1988), but no study has been comprehensive. Herein, we address the following questions for several populations of *E. crista-galli*, broadly spanning its distribution:

(1) What is its reproductive phenology?
(2) What are the flower duration, different floral phases, and the functional structure of the flower in relation to pollination?
(3) What is the nectary structure as well as the chemical composition of the nectar?
(4) What is the secretory pattern, the sugar composition of nectar for a single flower's duration, with respect to the nectar standing crop?
(5) What is the floral response to nectar removal?
(6) What is the breeding system of this species?
(7) Are there fruit differences produced by the different pollination treatments?
(8) Who constitutes floral visitors, what is the frequency of the visits, and what is their pollination role?

Material and Methods

Fieldwork was conducted on a total of 158 adult individuals from four natural populations and three cultivated ones (Table 1, Fig. 1). Cultivars were considered because there is high fruit and seed predation by beetles (Curculionidae, *Araptus sparsepunctatus*; Viana, 1965) in natural populations, obscuring data. Vouchers for all seven populations are deposited in CORD, UNR, and MVFA (Table 1).

Phenology data were recorded in 1994–1995 for five of the seven populations (see Results). Data included periods of bud production, major and additional flowering events, fruit production, and seed dispersal. In population ARG-ER1, a detailed study of 10 trees was performed at the same time.

Flowers from population ARG-ER1 were fixed in FAA, dehydrated through an ethyl alcohol/xylol series, and embedded in paraffin to study their anatomical structure (Conn et al., 1960). Sections in cross and longitudinal views were cut at 10-μm intervals and stained with safranin and astral blue (Johansen, 1940). To localize stomata or starch grains, nectary tissue was cleared with NaOH (10% aqueous solution), washed with acetic acid : water (1 : 3), spread on a slide, and stained with an aqueous I_2-IK solution (Johansen, 1940). Drawings were made using a camera lucida attachment to a Zeiss microscope. Photomicrographs were taken on a

Table 1. Study sites for *E. crista-galli* populations. Vouchers are housed at CORD (Galetto), UNR (Vesprini), and MVFA (Speroni).

Population abbreviation	Localities, vouchers (number of trees studied)	Latitude and longitude
ARG-ER1	Argentina. Entre Ríos Province, Dept. Victoria, Isla Charigüe, *Galetto 274, Vesprini s.n.* (41)	32°55′40″S 60°36′67″W
ARG-ER2	Argentina. Entre Ríos Province, Dept. Paraná, National Park Pre-Delta, *Galetto s.n.* (18)	32°05′36″S 60°40′48″W
ARG-Chaco	Argentina. Chaco Province, Dept. Capital, Resistencia, *Galetto 382* (7)	27°30′03″S 58°56′48″W
URU	Uruguay. Dept. Colonia, Balneario Astilleros, *Speroni s.n.* (80)	34°25′31″S 57°33′59″W
CULT-1	Cultivar. Argentina. Córdoba Province, Dept. Capital, Argüello, *Galetto 381* (3)	31°19′12″S 64°18′36″W
CULT-2	Cultivar. Argentina. Córdoba Province, Dept. Capital, Alta Córdoba, *Galetto 79* (2)	31°22′12″S 64°09′02″W
CULT-3	Cultivar. Argentina. Córdoba Province, Dept. Capital, Observatorio, *Galetto 380* (7)	31°27′00″S 64°12′01″W

Zeiss Axiophot microscope, using Kodak T-max film, 100 ASA.

Pollen and ovule numbers from 15 randomly chosen flowers from population ARG-ER1 were counted (1 to 3 flowers from seven trees). All ten anthers belonging to a single preanthesal flower were softened in 1 N HCl for 12 hours at room temperature, transferred to a known volume of lactic acid:glycerin (3 : 1) in a test tube, and macerated with a glass rod. The mixture was homogenized using a vortex mixer, and a sample of known volume of this solution was placed in a hemocytometer. The grains within six randomly chosen squares were counted, and the total number of grains per flower was then calculated. The ovary was directly dissected under a Zeiss stereo microscope and the ovules counted. Pollen stainability, as an indicator of pollen viability, was calculated with aniline blue (1% mass/vol) in 100 grains per flower, corresponding to sampling for pollen/ovule.

Experimental fieldwork was done from October to April for three reproductive seasons, 1993 to 1996. In all cases, flowers in bud stage were tagged for identification. They were bagged using paper bags to prevent pollinator visits and nectar robbers. Flower longevity was determined in three populations (ARG-ER1, URU, and CULT-1) for randomly chosen bagged flowers by following their development until they began to fade.

Nectar was extracted with capillary glass tubes without removing the flowers from the plant, avoiding damage to the nectaries. Two variables were immediately taken: volume (in µl) using graduated micropipettes and sugar concentration (%, mass/total mass) with a pocket refractometer (Atago, Japan). The amount of sugar produced was expressed in milligrams and was calculated after Kearns and Inouye (1993). In Table 3, dates, population identification, samples, individuals, and those flowers analyzed were recorded. Separation of sugars was by gas chromatography following methodology indicated in Bernardello et al. (1994). Sugar ratio (r) was calculated as sucrose/fructose + glucose (H. G. Baker & I. Baker, 1983) and the hexose ratio (hr) as glucose/fructose. Nectar samples for sugar analysis were collected from the four anthesal stages for flowers, from different populations, and from different periods within the flowering season. Tests for amino acids, lipids, phenols, alkaloids, and reducing acids were also performed on nectar drops placed on Whatman #1 chromatography paper (H. G. Baker & I. Baker, 1975). A histidine scale was used to quantify amino acids (H. G. Baker & I. Baker, 1975).

Nectar secretion pattern was determined during one reproductive season (1994–1995) in population ARG-ER1 (4 trees) and population CULT-1 (3 trees), using eight bagged-flower sets of 5 to 8 flowers in each population. Data were taken once for each set, allowing the nectar to accumulate until the measurement. The studied period covered the four days of the flower lifetime. Measurements were performed twice a day (two untouched new sets were used each day, at 8:00 and at 19:00 hr respectively).

The effect of nectar removal on total nectar production per flower was estimated on sets of 7 to 10 flowers within a population and on the same trees

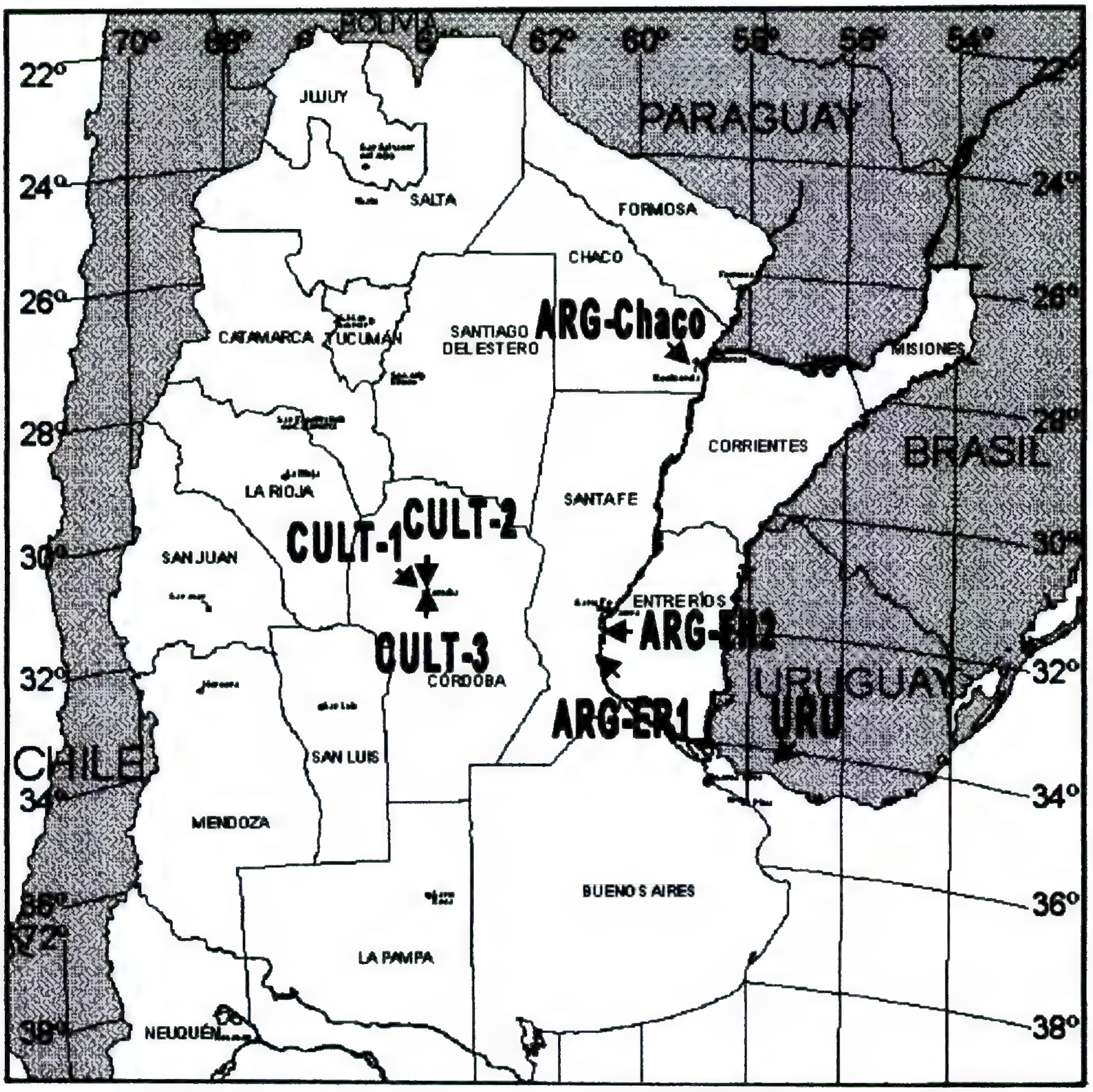

Figure 1. Location of the studied populations of *E. crista-galli*. Abbreviations: ARG-ER1 = Argentina, Entre Ríos, Victoria; ARG-ER2 = Argentina, Entre Ríos, Paraná; ARG-Chaco = Argentina, Chaco; URU = Uruguay, Colonia; CULT-1 to 3 = cultivars from Argentina, Córdoba. For more details, see Table 1.

as for nectar secretion. Nectar was removed and measured from the same flower twice at 8:00 and 19:00 hr throughout the first two days after flower opening. Set 1 was used as a control (flowers not yet involved within the resorption period). Thus, in order to compare the results accurately, sets were subjected to 1 to 4 measurements according to the scheme suggested by Torres and Galetto (1998). Data on the total mg of sugar produced by sets 1 to 4 were compared by a one-way analysis of variance (ANOVA), at the 0.05 significance level.

The standing crop of nectar was evaluated by measuring accumulated nectar (volume, concentration, and mg of sugar in the way described above) from individual flowers that had been fully exposed to pollinators. Data were randomly collected from open flowers (anthesal stages 3 and 4) from February to March 1996, sampling seven trees (N = 10 flowers/tree) from population ARG-ER1.

Different hand-pollinating treatments were applied to sets of 1- or 2-day-old flowers (N = 10–19). Data were taken in population ARG-ER1 (8 trees, 1994–1995/1995–1996), population URU (13 trees, 1994–1995), and population CULT-1 (3 trees, 1995–1996); the number of flowers used is included in Tables 5–7. Flowers were treated as follows: (1) autonomous self-pollination (buds bagged without treatment); (2) autogamous crosses (hand-pollinated flowers with pollen from their own dehisced anthers); (3) geitonogamous crosses (emasculated hand-pollinated flowers with pollen from flowers of the same ramet); (4) xenogamous crosses (emasculated hand-pollinated flowers with pollen from flowers belonging to a different genet

Table 2. Flowering, fruiting, and seed dispersal periods of *E. crista-galli*. Data taken in November 1994 to April 1995.

Population	Flowering (dates/period)		Fruiting (dates/period)	Seed dispersal (dates/period)
	Major	Additional		
ARG-ER1	11/02–12/01	12/30–03/11	12/01–03/11	01/21–03/11
URU	11/05–11/30	01/10–03/15	12/10–04/12	01/29–04/12
CULT-1 to 3	11/10–12/08	01/09–03/06	12/20–03/27	01/20–03/27

located at least 200 m away); (5) tests for apomixis (stigmas and anthers were clipped at flower opening); and (6) controls (flower sets were tagged and left open to flower visitors).

Fruit set was recorded 6 to 7 weeks after treatment with the following features measured: fruit length and mass, total seed mass per fruit, seed number, mean seed mass, and percentage of seed germination. Seed samples were placed in Petri dishes lined with filter paper and watered regularly at room temperature for two months. To obtain the seed germination percentage, the germinated seeds were counted and divided by the number of seeds used. Pre-emergent reproductive success or PERS (Wiens et al., 1987) was calculated as (# of fruits obtained/# of treated flowers) × (mean # of seeds per fruit/mean # of ovules per flower).

Flower visitors were identified during 1 to 3 flowering periods (1993–1996) in five populations (ARG-ER1, ARG-ER2, ARG-Chaco, URU, CULT-1) with an average of 49.8 hours of observation per population. Visitors were collected and/or photographed for identification. Relative abundance was quantified in population ARG-ER1 on one day. The number of individual visitors and flowers visited were recorded. The inflorescences were observed for a total of 7 hr., in 30-min. periods beginning at 06:00. All observations were made from a fixed spot from which 42 flowering branches of three trees could all be monitored.

Fruit quality traits were compared by *t*-tests or one-way analyses of variance (ANOVA) combined with Bonferroni test at $P < 0.05$. Analogous nonparametric methods (Mann-Whitney, Kruskal-Wallis, and Wilcoxon rank tests) were used when the assumptions required for parametric methods were not met (Sokal & Rohlf, 1995). The statistical program package SPSS (1992) was used for these analyses.

Results

Phenology

This species displayed a major flowering burst in November for all the studied populations (Table 2). Additionally, most of the trees presented one or two flowering episodes of lower amplitude in January through March. This second flowering was very variable among individuals and even among branches of the same tree. Detailed analysis on population ARG-ER1 showed that, although there is a general synchrony in flowering, fruiting, and seed dispersal periods within each population, there were some differences among the trees at the beginning and the peak of flowering (Fig. 2). The observed phenology corresponds to the sub-annual frequency class of Newstrom et al. (1994), with at least two cycles per year seen in spring and summer. Within the latitudinal boundaries of the study, this pattern appeared stable, although there were minor differences among the individuals within a population (Fig. 2).

Inflorescence and flower features

Flowers in *Erythrina crista-galli* are resupinate, red, odorless, diurnally open, lasting for 3 or 4 days. They were borne in racemes (pictured in Fig. 3A) with a mean of 51.67 ± 13.06 flowers per raceme (N = 46, 17 trees, populations ARG-ER1, ARG-Chaco, CULT-1, CULT-2). Raceme axes are generally erect, but may be pendulous or intermediate in position, principally on the lower branches. Anthesis proceeds acropetally and, as a result, there are many open flowers simultaneously in each raceme.

The calyces are bowl-shaped and fleshy. The standard is broad, elliptic, and rigid; the wings are asymmetric, very small, and concealed by the calyx, while the keel is falcate and acute at apex. Anthers and stigma may remain exposed at floral summit (Figs. 4, 5A). The androecium is pseudomonadelphous with 10 stamens, arranged as 9 longer + 1 shorter. The anthers are dorsifixed and introrsely dehiscent. The ovary is stipitate and pubescent with a glabrous style and capitate, moist stigma (Fig. 5A). Flowers average 15.25 ± 1.61 ovules and 878,485 ± 144,770 pollen grains (a pollen/ovule ratio of 57,605). Pollen viability is high (96.45% ± 2.70).

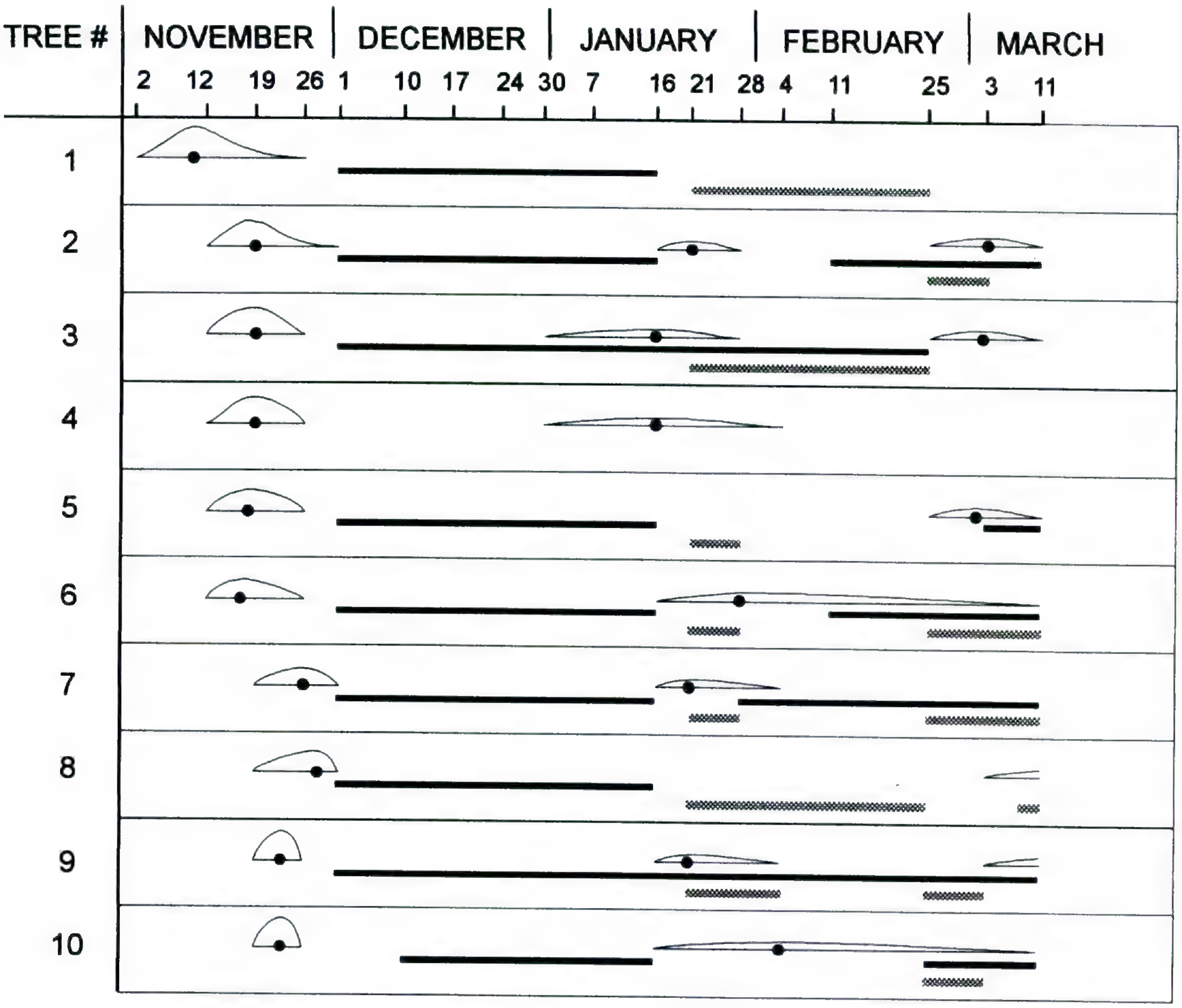

Figure 2. Flowering, fruiting, and seed dispersal periods in *E. crista-galli*, taken for 10 trees of population ARG-ER1 (November 1994 to March 1995). Dots indicate qualitative flowering peak.

Floral ontogeny has been subdivided into four stages, as illustrated in Figure 4: (1) 4–5-cm preanthesal bud ready to open, with the standard completely folded and occluding floral parts (time: 0 hr); (2) corolla standard beginning to unfold from its base, initiating the anthesis (first day, time: 3 to 12 hr); (3) fully open flower, 5–6 cm long with standard entirely spread (the degree of spreading of the standard is variable, reaching its maximum when it is perpendicular to the keel; 2nd and 3rd days, time: 24 to 36 hr); and (4) 3- to 4-day-old flowers beginning to fade (3rd and 4th days, time: 36 to 64 hr). Flowers of most populations typically went through all these stages. However, many flowers from population ARG-ER1, and many of population URU, remained in stage 2, i.e., the standard did not open spontaneously; in this case, the flower lifetime was also 3 to 4 days.

NECTARY

A structural floral nectary is located in the receptacle between stamens and ovary (Fig. 5), and is supplied by vascular bundles having both phloem and xylem (Fig. 6). It is a small ring, slightly divided at the apex into 10 lobules. Histologically, the external part of the ring is composed of 10 to 14 layers of secretory tissue with small cells highly stained. In contrast, the internal ring portion is composed of 22 to 26 parenchymatous layers with

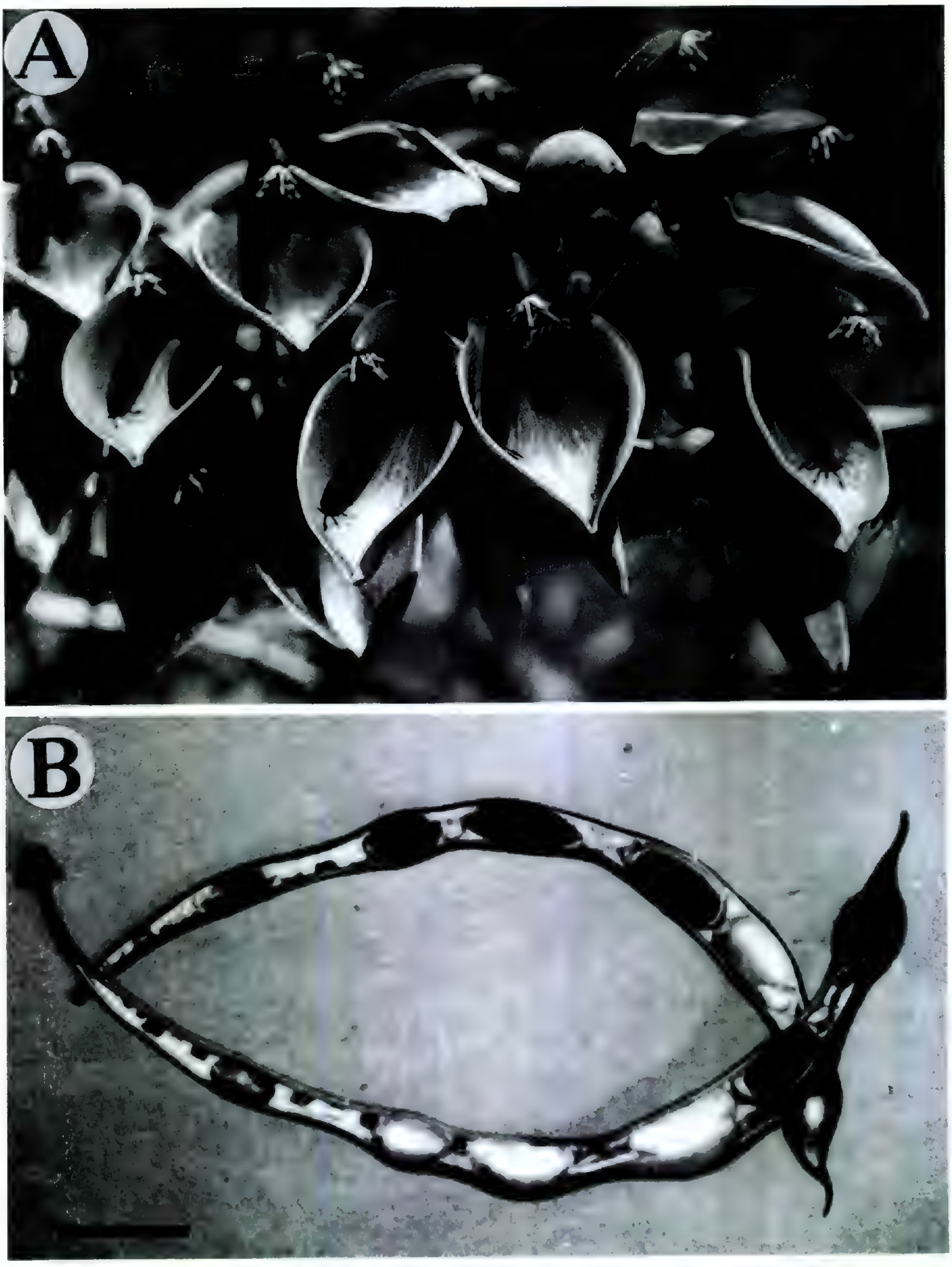

Figure 3. Inflorescence and fruit photographs of *E. crista-galli* from population URU. —A. Partial view of an inflorescence. —B. Dehiscent fruit showing seeds. Both bars are equivalent to 2 cm.

larger, less stained cells, and the vascular bundles (Fig. 6). Stomata are found mainly in the upper part of the lobules. The basal part of the fused staminal filaments delimits a nectariferous chamber where the exudate accumulates, protected from evaporation.

NECTAR CHEMICAL COMPOSITION

Flowers produced nectar with a mean sugar concentration of 21.8% (Table 3). Nectar concentration was consistent among the different populations sampled (Table 3). Nectar sugars were glucose, fructose, and sucrose. Mean percentage of sucrose was lower than 2% or absent, whereas the percentage of hexose sugars was higher than 98%, and always with more glucose than fructose (Table 3). All samples had amino acids in variable concentrations (from traces to 1.9 mg/ml, i.e., 9 in the histidine scale). Samples from Uruguay (population URU) had the lowest amount, while the ones from population ARG-ER1 had the highest (Table 3). Phenols were always detected, whereas alkaloids, reducing acids, and lipids were not. In population

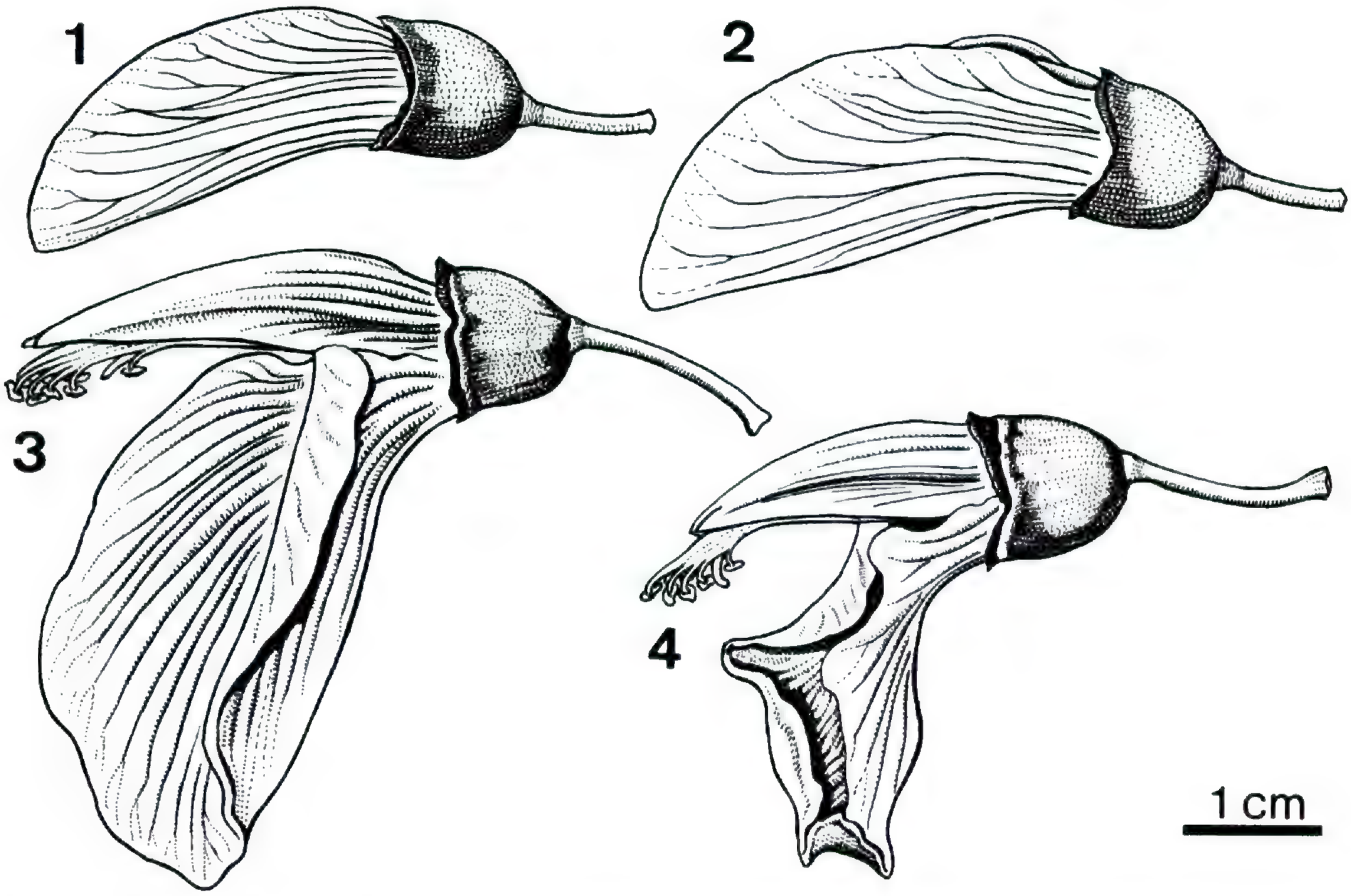

Figure 4. Flower stages of *E. crista-galli* as indicated in the text. —1. Preanthesal bud. —2. Bud initiating anthesis (1-day-old flower). —3. Open flower (2- to 3-day-old flower). —4. Old flower beginning to fade (3- to 4-day-old flower).

CULT-1, we analyzed the sugar composition throughout the flower lifetime (Table 4). The sugar proportions data obtained for the different flower ages also showed a remarkable constancy throughout flower lifetime (Table 4).

NECTAR PRODUCTION AND RESORPTION

This was studied in two populations from different habitats: a natural one growing in a very humid environment (ARG-ER1), and a cultivated one from

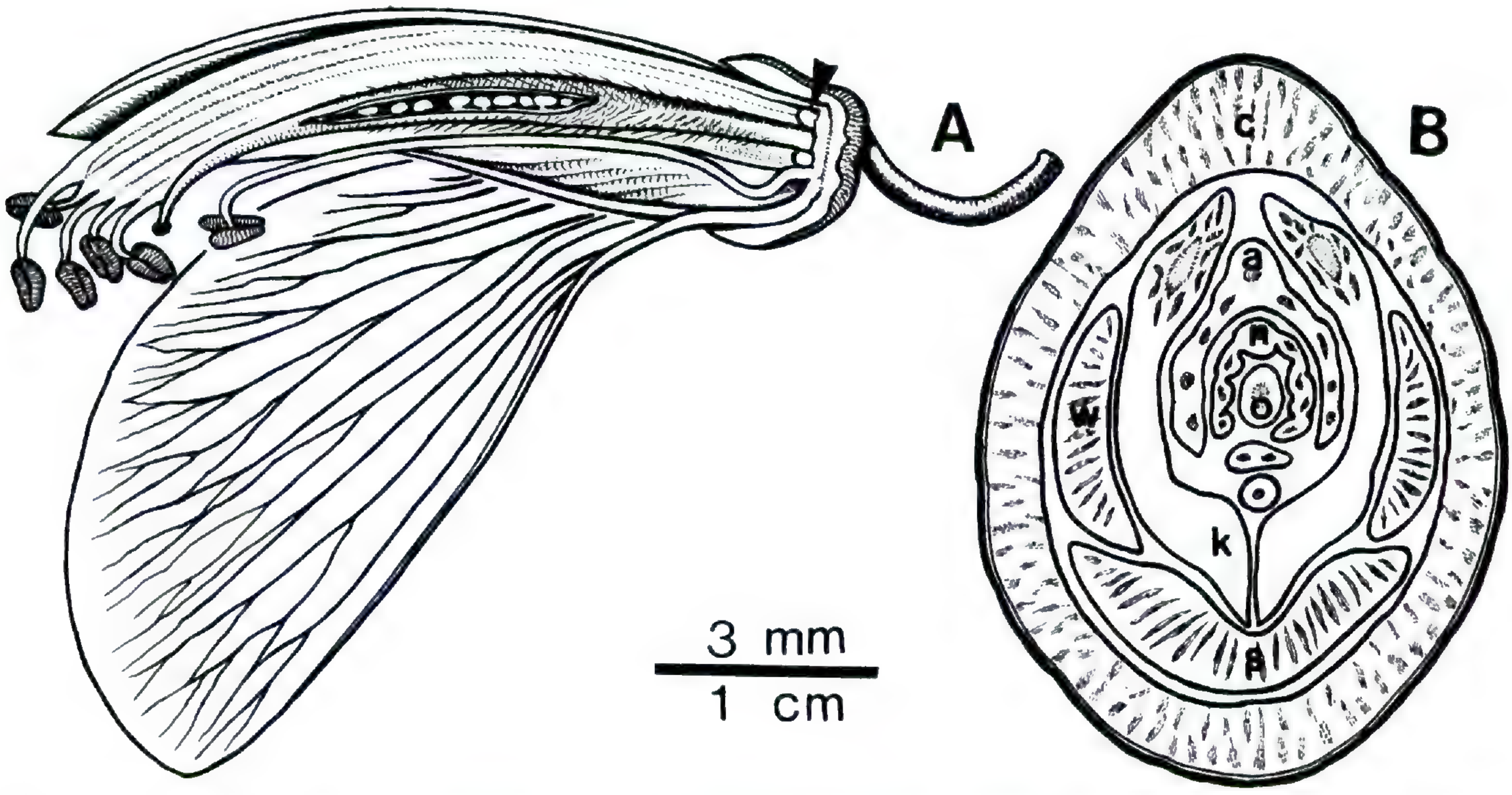

Figure 5. Open flower of *E. crista-galli.* —A. Longisection showing nectary location (arrow head). —B. Flower transection at the nectary level. Abbreviations: c = calyx, s = standard, w = wing, k = keel, a = androecium, n = nectary, o = ovary. 1-cm bar for A; 3-mm bar for B.

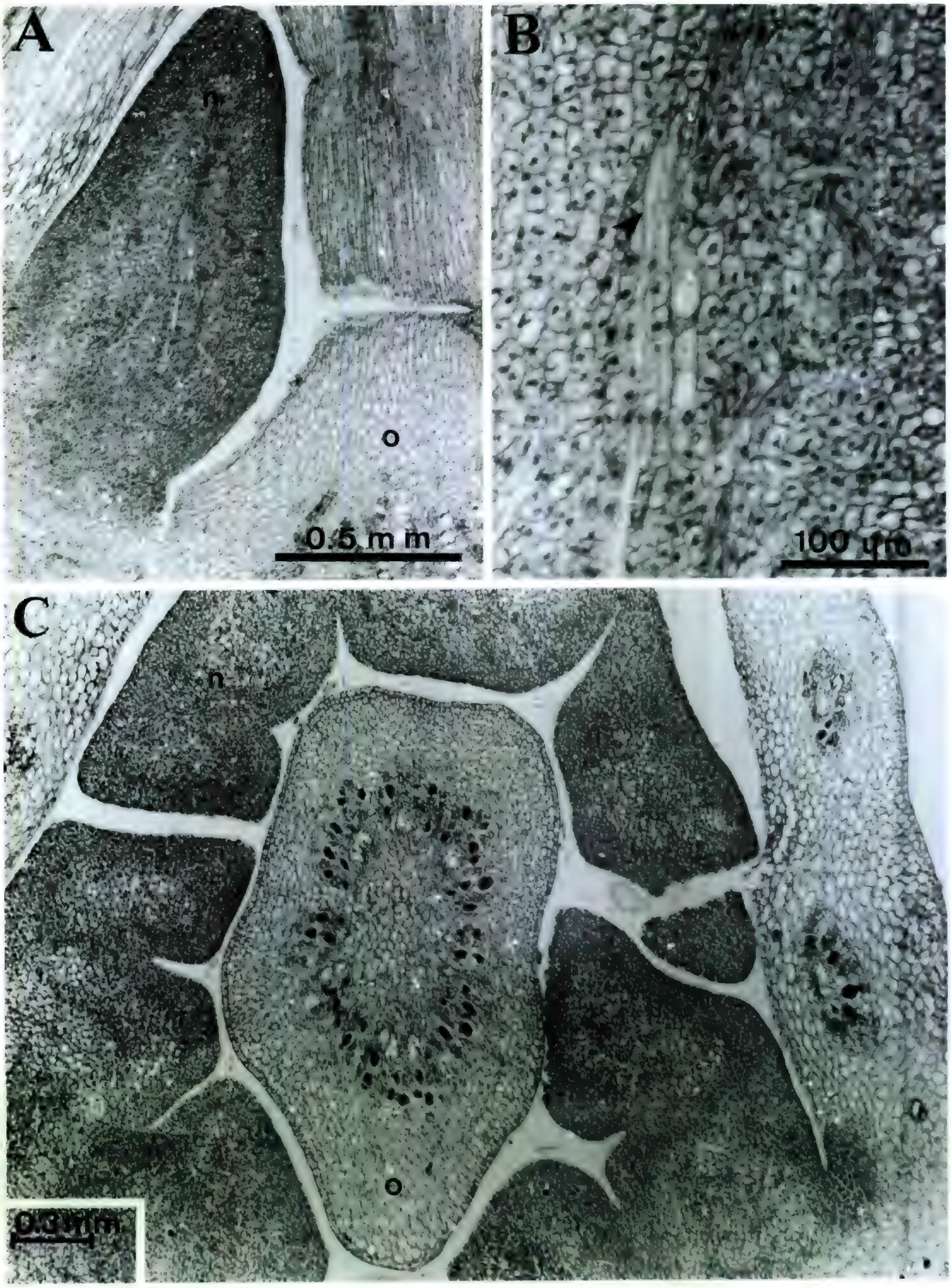

Figure 6. Optical microscope photomicrographs showing nectary structure in *E. crista-galli*. —A. Flower partial longitudinal section. —B. Detail of the central nectary tissue with the vascular bundle indicated by arrow. —C. Flower partial transection at the level of nectary base. Abbreviations: n = nectary, o = ovary.

Table 3. Nectar chemical composition of *E. crista-galli*. N = number of individuals (number of flowers) from which nectar was sampled. Conc. = nectar concentration (mass/total mass). HS = histidine scale to quantify amino acids (H. G. Baker & I. Baker, 1975). S/(F + G) = sugar ratio, G/F = hexose ratio, t = traces.

Population	Date	N	Conc. %	HS	Sugars (mean ± sd; %, mass/mass)			Ratios	
					Sucrose	Fructose	Glucose	S/(F + G)	G/F
ARG-ER1	Dec-94	1 (9)	22.0 ± 1.1	8	1.0 ± 0.2	31.5 ± 2.8	67.5 ± 3.0	0.009	2.14
ARG-ER1	Mar-95	2 (10)	21.4 ± 0.7	6	0.0	41.9 ± 1.1	58.1 ± 1.1	0.0	1.41
ARG-ER1	Mar-96	3 (13)	19.5 ± 0.8	8	0.2 ± 0.1	36.0 ± 1.1	63.8 ± 1.0	0.001	1.77
ARG-ER2	Nov-95	1 (10)	24.3 ± 1.6	8	1.2 ± 0.3	36.1 ± 1.0	62.7 ± 1.4	0.012	1.74
ARG-ER2	Dec-95	1 (15)	21.5 ± 1.2	9	0.0	36.0 ± 2.6	64.0 ± 2.6	0.0	1.78
ARG-ER2	Jan-96	1 (7)	20.2 ± 0.3	7	0.0	35.2 ± 1.3	64.8 ± 1.3	0.0	1.84
AGR-Chaco	Jan-96	4 (22)	22.5 ± 0.4	6	0.9 ± 0.1	30.7 ± 0.7	68.4 ± 0.7	0.009	2.23
URU	Dec-96	1 (10)	21.2 ± 1.1	t	0.0	30.6 ± 2.7	69.4 ± 2.7	0.0	2.27
URU	Jan-95	1 (15)	23.4 ± 1.8	9	0.0	28.2 ± 2.9	71.8 ± 2.9	0.0	2.54
URU	Jan-95	1 (19)	19.9 ± 1.1	1	0.0	37.4 ± 1.1	62.6 ± 1.1	0.0	1.67
CULT-1	Dec-95	3 (12)	24.0 ± 0.6	7	0.0	38.1 ± 1.7	61.9 ± 1.7	0.0	1.62
CULT-2	Jan-96	2 (16)	23.2 ± 0.5	7–8	0.1 ± 0.1	36.9 ± 2.2	63.0 ± 2.1	0.001	1.70
CULT-3	Feb-96	4 (17)	19.8 ± 0.9	7	0.8 ± 0.5	35.1 ± 0.3	63.9 ± 0.6	0.008	1.82
Mean of means			21.8 ± 1.6	6.4	0.3 + 0.5	34.9 ± 3.7	64.8 ± 3.7	0.003	1.88

a drier habitat (CULT-1). Most of the total nectar was secreted by buds. As soon as the flowers opened, nectar was available to pollinators (Fig. 7). Some differences were seen between the flowers of the two analyzed populations. In population ARG-ER1, the flower nectar volume was higher (Fig. 7A), the nectar concentration lower (Fig. 7B), and accordingly, the amount of sugar secreted by a flower was comparable (Fig. 7C). With respect to nectar secretion pattern, concentration remained unchanged with a little decrease at the end of the flower lifetime in both populations. In contrast, some differences between the populations were found for flower nectar volume and sugar mass patterns. In population CULT-1, there was a short nectar cessation period after flower opening which lasted for 12 hours, followed by a new nectar secretion period on the first night. Only then did the resorption phase begin (Fig. 7A, C). In population ARG-ER1, most of the nectar was secreted in bud stage (Fig. 7A, C). Then flowers showed two alternate short periods of resorption and secretion until the third day, whereupon a final resorption period began (Fig. 7A, C). The nectar resorption rate was similar in both populations: −0.38 mg/hr. in population ARG-ER1, −0.31 mg/hr. in population CULT-1.

NECTAR REMOVAL

Figure 8 shows the amount of sugar produced by sets subjected to different numbers of removals in the two studied populations. Very little or no nectar was removed at consecutive intervals (Fig. 8). One-way ANOVA results indicate that removal had no effect on nectar production, since there were not significant differences between the treated flower sets (population ARG-ER1: $F_{[4, 49]} = 2.22$, $P =$

Table 4. Nectar sugar composition throughout flower lifetime for bagged flowers not visited by pollinators of *E. crista-galli*. Data taken on sets of flowers (n = 8 flowers each) from population CULT-1. Sugar ratio = S/(F + G), Hexose ratio = G/F.

Flower stage	Sugars (mean ± sd; %, mass/mass)			Ratios	
	Sucrose	Fructose	Glucose	S/(F+G)	G/F
Bud	1.2 ± 0.3	37.3 ± 1.0	61.6 ± 1.3	0.010	1.65
1st day	0.0	34.5 ± 1.5	65.9 ± 0.5	0.0	1.91
2nd day	1.9 ± 0.8	40.6 ± 1.3	57.5 ± 0.9	0.020	1.41
3rd day	1.8 ± 0.6	37.1 ± 1.2	61.1 ± 1.9	0.019	1.64
4th day	1.7 ± 1.2	35.4 ± 0.9	62.9 ± 2.3	0.017	1.77
Mean of means	1.3 ± 0.7	36.9 ± 2.1	61.8 ± 2.8	0.013	1.67

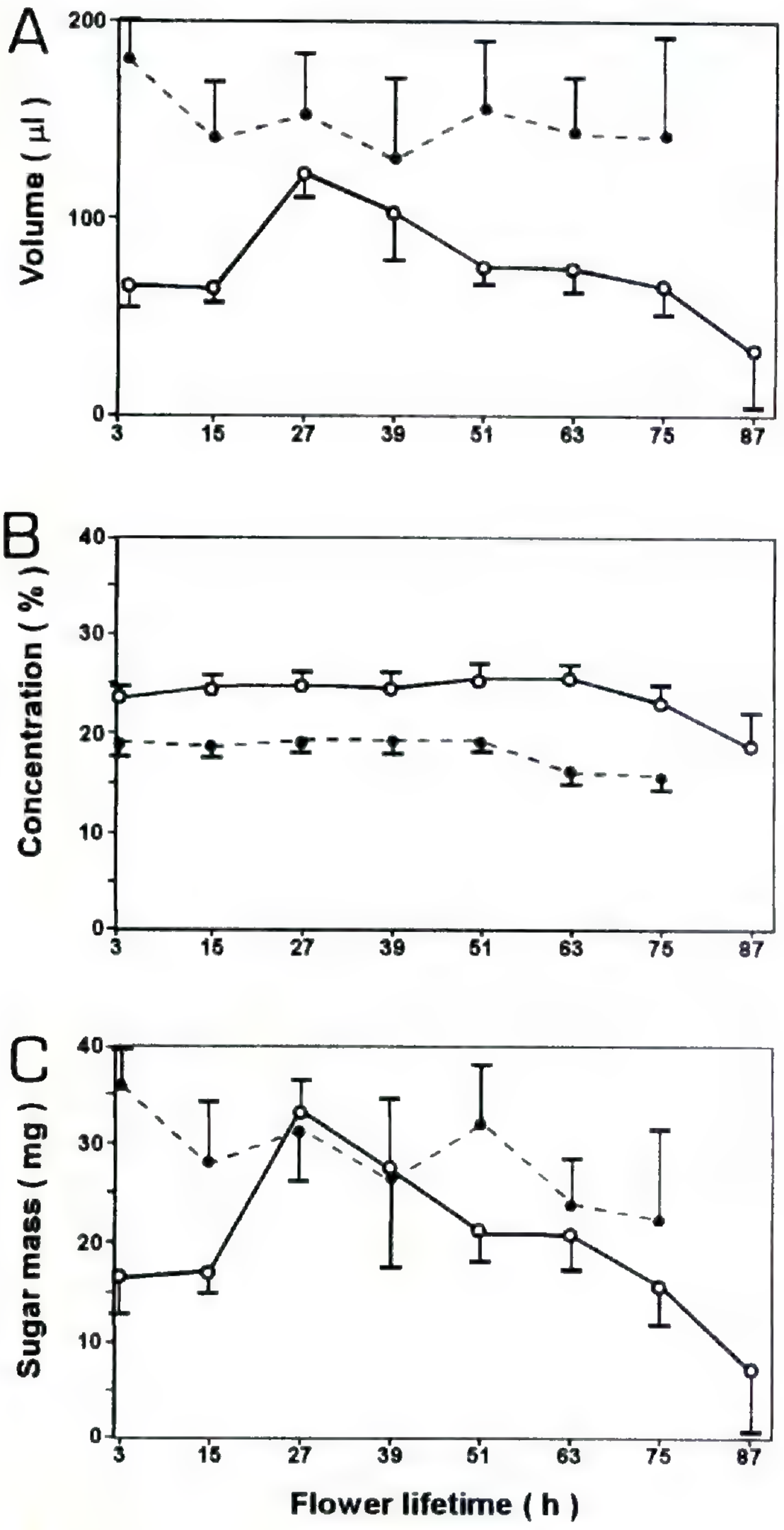

Figure 7. Nectar production of *E. crista-galli* throughout flower lifetime. Data correspond to nectar volume per flower (µl), sugar concentration in nectar (%, mass/mass), and sugar mass (mg) per flower. Population ARG-ER1 = broken line, black circles; population CULT-1 = solid line, white circles. Plotted values represent the mean + 1 s.d. and correspond to a sample size of 5 to 8 bagged flowers each.

0.08; population CULT-1: $F_{[3, 25]} = 0.45$, $P = 0.13$). Concomitantly, there were no significant differences in mean total amount of nectar secreted per flower at the population level ($t = 1.36$, $P = 0.18$).

NECTAR STANDING CROP

Flower average values of nectar standing crop were obtained on seven trees from population ARG-ER1. One-way ANOVA comparisons showed no significant differences among the sampled trees (mg of nectar sugars: $F_{[6, 87]} = 0.79$, $P = 0.58$; nectar volume: $F_{[6, 87]} = 0.24$, $P = 0.96$). Nectar data from open flowers significantly differed from that obtained from bagged 1- or 2-day-old flowers: volume (36.4 ± 39.8 and 158.3 ± 36.0, respectively; $U = 126.5$, $P < 0.00001$) and milligrams of sugar (7.4 ± 8.6 and 31.1 ± 7.8, respectively; $U = 184$, $P < 0.00001$). However, they did not differ with respect to concentration (17.7 ± 5.8 and 18.3 ± 1.4, respectively; $U = 1543$, $P = 0.06$).

BREEDING SYSTEM

Hand-pollination tests confirmed *Erythrina crista-galli* to be self-compatible (Table 5). Artificial autogamous crosses were more successful than geitonogamous or xenogamous ones (Table 5). However, spontaneous autogamy very rarely occurred: data from two populations showed that less than 1% of the flowers set fruit in this way (Table 5). Natural fruit set was always lower than fruit set from hand crosses, except for population ARG-ER1 (Table 5).

FRUIT AND SEED CHARACTERISTICS

These legume pods are brown, arcuate, and coriaceous to subligneous, dehiscing by the two margins (Fig. 3B), and presenting 1 to 9 seeds. The seeds are brown mottled with black, dry, and hard. In spite of low natural fruit set (< 6%, Table 5), individuals often yielded thousands of seeds because of the large number of both flowers produced per plant, and seeds produced per fruit (Table 6).

Fruit set obtained by geitonogamous hand-pollination was the lowest and significantly different from autogamous hard-pollination, but not from fruit set obtained by hand cross-pollination (Table 6). Reproductive success for geitonogamy was also the lowest but the differences from the other pollination treatments were not significant. Fruits obtained by hand-pollination treatments from population CULT-1 were compared in their quantity (Table 6). All variables showed significant differences, except for seed number per fruit. Highest values for fruit length and mass, seed number and seed mass per fruit, mean seed mass, and seed germination percentage were observed in xenogamous fruits and seeds (Table 6). In contrast, the lowest mean seed mass and the lowest seed germination percentage were seen for autogamous seeds (Table 6).

When unbagged-pollinated fruits were compared, natural populations were variable. Significant differences were found for fruit length and mass, seed number and seed mass per fruit, mean seed mass, and seed germination percentage (Table 7). Fruits from cultivated trees (populations CULT-1 to 3) showed the highest values for most variables

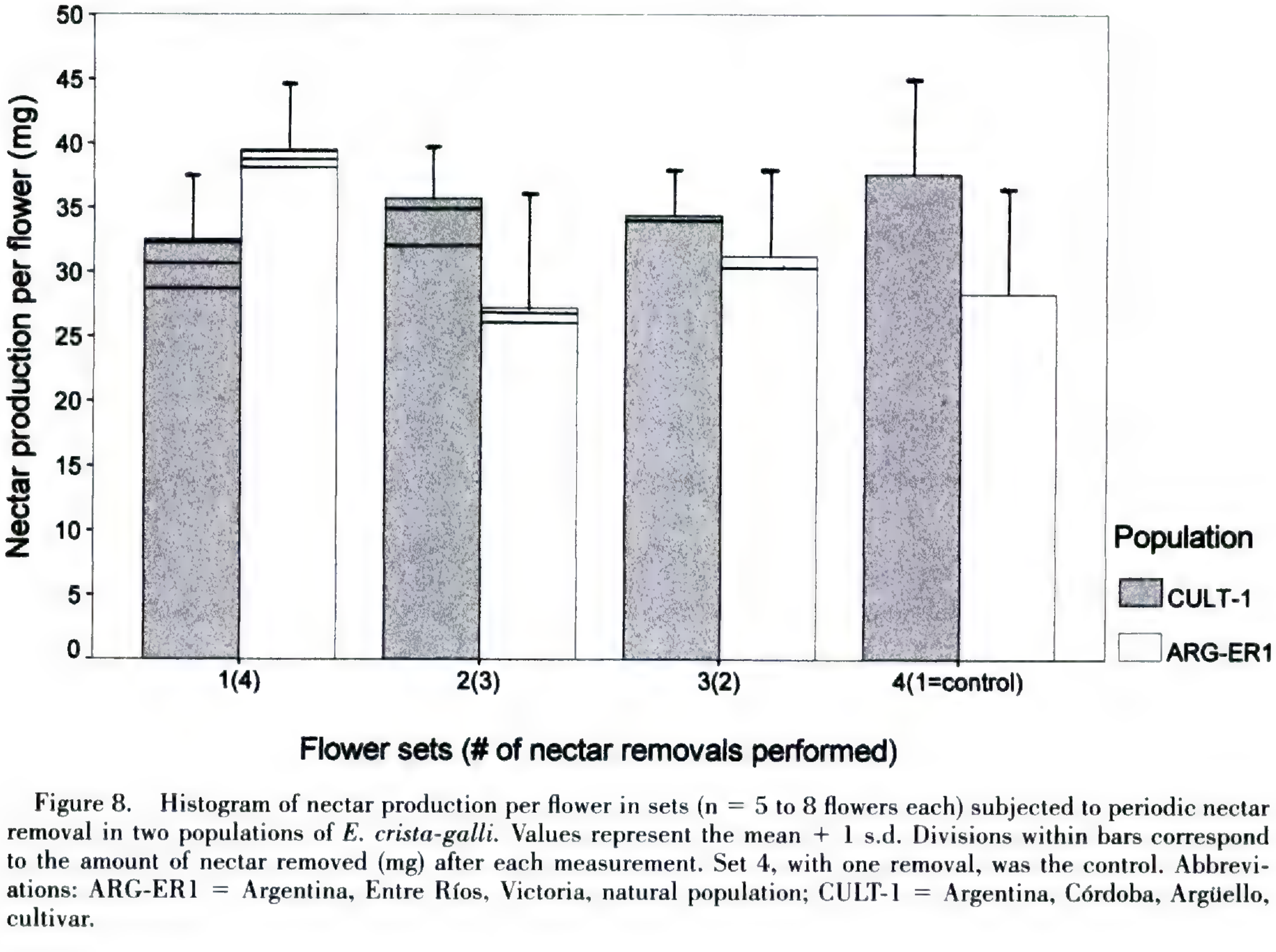

Figure 8. Histogram of nectar production per flower in sets (n = 5 to 8 flowers each) subjected to periodic nectar removal in two populations of *E. crista-galli*. Values represent the mean + 1 s.d. Divisions within bars correspond to the amount of nectar removed (mg) after each measurement. Set 4, with one removal, was the control. Abbreviations: ARG-ER1 = Argentina, Entre Ríos, Victoria, natural population; CULT-1 = Argentina, Córdoba, Argüello, cultivar.

of fruits and seeds (i.e., fruit mass, seed number and seed mass per fruit, mean seed mass, and seed germination percentage; Table 7).

Germination percentages of seeds from open-pollinated fruits were similar to those obtained for experimental autogamous fruits, but were considerably lower than those of geitonogamous and xenogamous fruits (Tables 6, 7).

VISITORS

Animals visiting the flowers for nectar were documented only during daylight hours. Visitor activity was noted as low or absent in rainy, windy conditions, or during the heat of midday (> 30°C). Hymenopterans and hummingbirds consistently visited the populations of these trees in most of the areas sampled (Table 8, Fig. 9B–H). The most common insects were carpenter bees (*Xylocopa nigrocincta*, Fig. 9C, *X. ordinaria*, Fig. 9D, and other *Xylocopa*) as well as honeybees (*Apis mellifera*, Fig. 9G, H). Four species of hummingbirds were noted (Table 8), with *Hylocharis chrysura* and *Chlorostilbon aureoventris* (Fig. 9B) most frequently observed. One species of perching birds (the epaulet oriole, Emberizidae, *Icterus cayanensis*, Fig. 9A) was occasionally seen in population ARG-ER2. Flies, butterflies, wasps, ants, and beetles were occasionally seen as incidental flower visitors (Table 8).

Table 5. Fruit sets by hand and naturally pollinating treatments in *E. crista-galli*. Numbers in parentheses give the number of flowers treated.

Treatment	Population			Total fruit set
	ARG-ER1	URU	CULT-1	
Autogamy (by hand)	0.024 (166)	0.108 (37)	0.308 (120)	0.139 (323)
Geitonogamy (by hand)	0 (104)	0.086 (35)	0.100 (80)	0.050 (219)
Xenogamy (by hand)	0.026 (115)	0.081 (37)	0.197 (76)	0.092 (228)
Autonomous self-pollination	0.006 (452)	no data	0.014 (145)	0.008 (597)
Natural pollination	0.126 (174)	0.036 (2683)	0.132 (1245)	0.054 (4102)

Table 6. Reproductive success and fruit and seed parameters from different hand-pollination treatments in *E. crista-galli* from population CULT-1. Except for fruit set and reproductive success, data are means ± s.d. Lowercase letters as superscripts indicate *a posteriori* test results. Reproductive success = (# of fruits obtained/# of treated flowers) × (mean # of seeds per fruit/mean # of ovules per flower), ns = not significant, ** = $P < 0.01$, *** = $P < 0.001$.

Variable	Autogamy	Geitonogamy	Xenogamy	Statistical tests
Fruit set (flowers treated)	0.308^{a} (120)	0.100^{b} (80)	0.197^{ab} (76)	$\chi^2 = 10.76$**
Fruit length (cm)	17.8 ± 2.9^{a}	14.4 ± 3.5^{b}	22.8 ± 1.9^{a}	$F_{[2, 36]} = 13.39$***
Fruit mass (g)	2.5 ± 0.9^{a}	2.2 ± 1.0^{a}	4.3 ± 0.6^{b}	$F_{[2, 36]} = 10.29$***
Total seeds per fruit mass (g)	1.2 ± 0.6^{a}	1.1 ± 0.6^{a}	2.0 ± 0.7^{b}	$F_{[2, 36]} = 4.79$**
Ovule number	15.1 ± 2.0	14.0 ± 0.8	13.3 ± 1.3	$F_{[2, 36]} = 1.73$ ns
Seed number per fruit	4.5 ± 1.8	3.3 ± 1.6	5.9 ± 2.1	$F_{[2, 36]} = 3.12$ ns
Mean seed mass (mg)	26.3 ± 6.2^{a}	33.4 ± 11.3^{b}	34.2 ± 10.1^{b}	$F_{[2, 36]} = 6.08$**
Seed germination (%)	49.50 ± 17.2^{a}	88.2 ± 12.0^{b}	93.3 ± 7.2^{b}	$F_{[2, 18]} = 12.58$***
Reproductive success	0.092	0.023	0.087	$\chi^2 = 4.38$ ns

Monkeys (Cebidae, *Allouata caraya*) were observed in population AGR-Chaco breaking and eating flowers. Although destructive, these actions may produce some accidental pollinations.

Visitor frequency was recorded only for population ARG-ER1. Almost 93% of the flowers registered were visited by hymenopterans (42.9 and 49.9% for honeybees and carpenter bees, respectively) with the remainder by hummingbirds (7.2%). Considering flower number of a tree visited by a single individual, carpenter bees and hummingbirds averaged a mean of 5 flowers per tree (4.7 ± 2.9 and 5.0 ± 2.5, respectively). By contrast, honeybee visits were more numerous, averaging over 21 flowers per tree (21.3 ± 7.5).

Hummingbirds and bees can be considered effective pollinators, because they contacted both anthers and stigma while taking nectar (Fig. 9B–G). However, we did not compare their effectiveness. Regardless of the flower visitor, the average time elapsed in each visit was 10 to 30 seconds per flower (range: 1–50 seconds). They usually visited many open flowers of several inflorescences in each tree before going to the next one. The remaining visitors were nectar thieves, because they did not contact both fertile whorls due to their small size. In population ARG-ER1, holes made by unknown primary nectar robbers were detected; these holes were used by small wasps to take nectar.

Our observations indicate that hummingbirds were widespread visitors while orioles were rare and local, but bees were even more frequent than hummingbirds. Hummingbirds usually foraged in the upper branches of the trees, taking nectar by inserting their bill between the standard and keel while touching anthers and stigma with their heads (Fig. 9B). *Chlorostilbon aureoventris* showed territorial behavior in population ARG-ER2.

Carpenter bees displayed a particular behavior recorded for all populations of *E. crista-galli*. They usually forced open preanthesal flowers (stage 2, Fig. 4) with their proboscide. This was of significance in the flowers of populations ARG-ER1 and URU that did not display natural flower opening. When these insects visited the flowers, they first opened them moving downward between the standard and the keel. Then, while looking for the nec-

Table 7. Reproductive characters from open-pollinated experiments in *E. crista-galli* populations. Nd = no data. Data represented by mean ± s.d. Lowercase letters as superscripts indicate *a posteriori* test results. *** = $P < 0.001$.

Variable	Population ARG-Chaco (n = 33)	Population URU (n = 40)	Population CULT-1 to 3 (n = 35)	Statistical tests
Fruit length (cm)	20.6 ± 4.2^{a}	16.7 ± 7.2^{b}	18.0 ± 3.0^{ab}	$F_{[2, 107]} = 7.16$***
Fruit mass (g)	1.6 ± 0.8	Nd	2.9 ± 1.0	$t = 5.6$***
Total seeds per fruit mass (g)	0.7 ± 0.4	Nd	1.6 ± 0.7	$t = 7.4$***
Ovule number	16.9 ± 1.7	Nd	15.2 ± 1.4	$t = -4.3$***
Seed number per fruit	3.4 ± 1.6^{a}	2.4 ± 2.6^{b}	4.5 ± 1.8^{a}	$F_{[2,107]} = 12.23$***
Seed mass (mg)	21.7 ± 5.5^{a}	26.6 ± 8.3^{b}	38.5 ± 8.1^{c}	$F_{[2,106]} = 52.63$***
Seed germination (%)	37.0 ± 22.5	Nd	52.1 ± 15.0	$t = 2.79$***

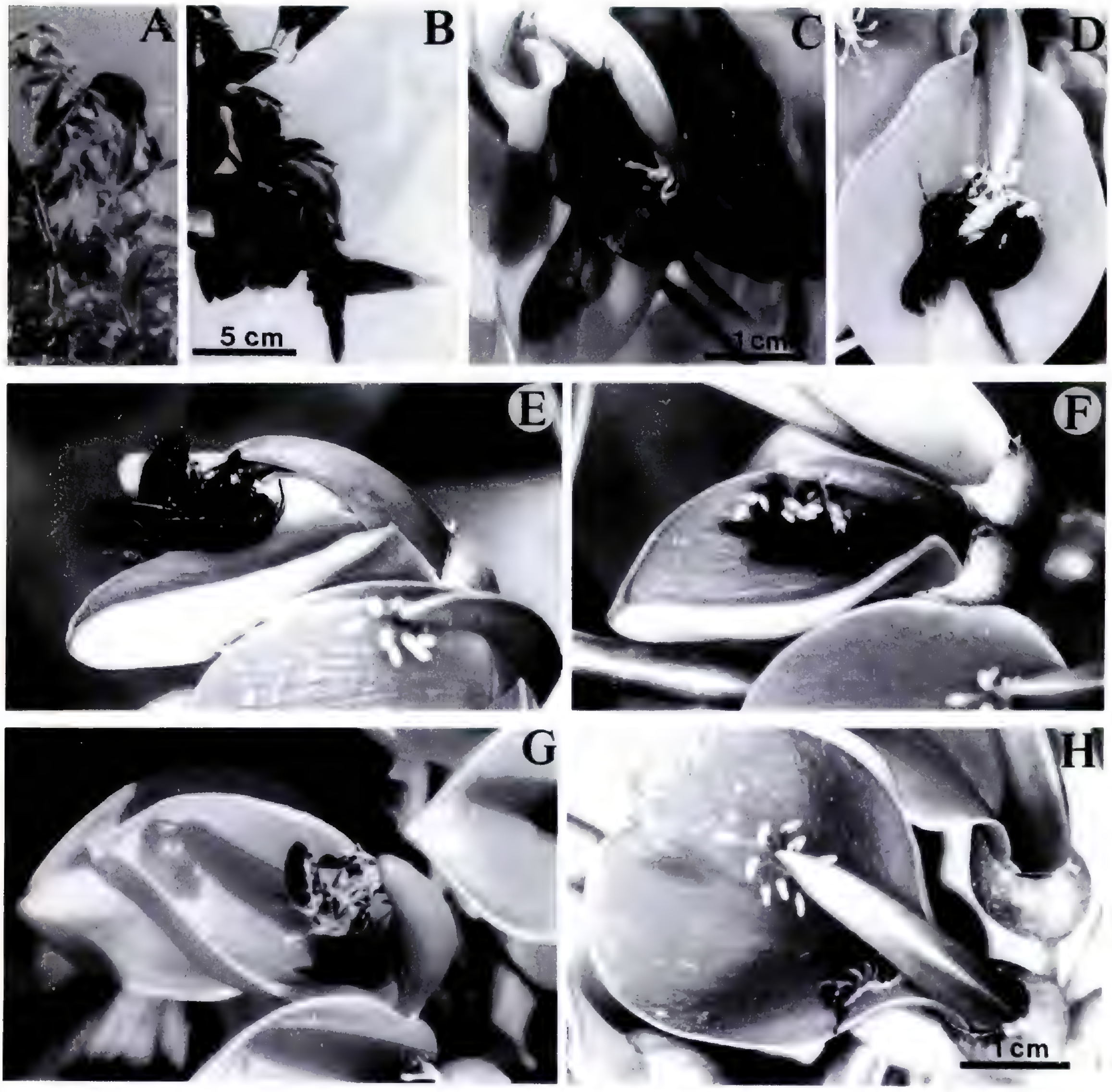

Figure 9. Floral visitors of *E. crista-galli*. —A. Oriole [*Icterus cayanensis* (Viellot)] from population ARG-ER2. —B. Hummingbird [*Chlorostilbon aureoventris* (D'Orbigny & Lafresnaye)] from population CULT-1. —C. Carpenter bee (*Xylocopa nigro-cincta* Smith) from population URU. —D. Carpenter bee (*Xylocopa ordinaria* Smith) from population CULT-1. —E, F. Bumblebee (*Bombus morio* Swed) from population CULT-1. —G, H. Honeybee *Apis mellifera* L. from populations URU and CULT-1, respectively. The 1-cm bar in C is valid for C–G. Abbreviations: ARG-ER2 = Argentina, Entre Ríos, Paraná; URU = Uruguay, Colonia; CULT-1 = Argentina, Córdoba, Argüello, cultivar.

tar, they touched the anthers and the stigma with their back, transferring the pollen.

Apis mellifera (Fig. 9G, H) was one of the most frequent visitors in all the populations, mainly during peak blooming. They occasionally forced preanthesal flowers. Honeybees are the only pollinators that collected pollen, in addition to nectar, mainly early in the morning from 7:00 to 9:00 hr. Landing on the apex of the androecium, they collected pollen, moving around the anthers and touching the stigma with their pollen-laden heads and forelegs (Fig. 9G). Then, they moved downward in the flower to take nectar (Fig. 9H). The average time they spent in each flower was 4 to 10 seconds.

Discussion

The flowers of *Erythrina crista-galli* present a nectary type characteristic of Papilionoideae (e.g., Fahn, 1979; Davis et al., 1988, and references therein). Nectar secretion possibly occurs via the modified stomata of the nectary through which nectar flows. The presence of stomata is a common feature of floral nectaries of legumes (Davis et al.,

Table 8. Flower visitors observed at *E. crista-galli* during the day.

	Population	ARG-ER1		ARG-ER2	ARG-Chaco	URU		CULT-1 to 3
	Sample	1	2	3	4	5	6	7
Visitors	Hours (days)	18 (6)	7 (1)	44 (11)	4 (2)	10.5 (3)	60 (10)	18 (6)
Trochilidae								
Chlorostilbon aureoventris (D'Orbigny & Lafresnaye)		X	X	X		X	X	X
Hylocharis chrysura (Shaw)		X	X	X		X	X	
Heliomaster furcifer (Shaw)			X					
Leucochloris albicollis (Viellot)						X	X	
Hymenoptera								
Anthophoridae								
Xylocopa nigro-cincta Smith		X	X			X	X	
Xylocopa ordinaria Smith								X
Xylocopa sp.			X	X				
Apidae								
Bombus morio Swed								X
Apis mellifera L.		X	X	X		X		X
Vespidae								
Polybia scutellaris White						X		
Polybia sericea Olivier						X		
Brachygastra lecheguana Latreille		X						
Vespoidea (nº species)				2	2			
Formicidae (nº species)		3				2	2	
Lepidoptera								
Pieridae (nº species)		3			3			
Diptera								
Muscidae (nº species)		1						
Syrphidae (nº species)		3						
Coleoptera								
Diabotrica speciosa Germ.		X						

1988, and references therein). Studies on the nectary stomata of *Vicia faba* L. demonstrated that their major functions seem to be those of assisting nectar escape from the gland and perhaps enhancing reabsorption of uncollected nectar (Davis & Gunning, 1993). *Erythrina crista-galli* secreted dilute and hexose-dominant nectar, with a remarkable constancy in its sugar proportions within the sampled area. Nevertheless, when comparing our data with previous studies, some differences appear in the nectar sugar composition. I. Baker and H. G. Baker (1979) noted traces of melezitose and maltose, whereas Gottsberger et al. (1984) found a much higher amount of sucrose (ca. 45%). This would indicate that there is some intraspecific variation in nectar sugar proportions, particularly when data from the northern Brazilian population are considered (22°45′S, 48°25′W; cf. Gottsberger et al., 1984).

With respect to nectar secretion pattern, there were some differences between the analyzed populations. Habitats are assumed to play a role in the differences found in nectar secretion between the populations, since trees from Entre Ríos (population ARG-ER1) live near the Paraná river, a more humid habitat than that of trees from Córdoba (population CULT-1). The fact of presenting most nectar at flower opening is unusual and unknown for other *Erythrina* species. As far as we know, it had been recorded only for some Indian bird-pollinated Loranthaceae (Davidar, 1983) and for the South American bee-pollinated *Mandevilla pentlandiana* (A. DC.) Woodson (Apocynaceae, Torres & Galetto, 1998). On the other hand, the observed decrease

in nectar volume and solute in 3-day-old bagged flowers can be inferred as resorption, based upon the constancy of nectar concentration throughout anthesis. There are some species of other families with flowers that last 4 days, with a similar nectar secretion-resorption pattern, and which are visited by a great variety of flower visitors (Combretaceae: Bernardello et al., 1994; Loranthaceae: Rivera et al., 1996; Apocynaceae: Torres & Galetto, 1998).

Most previous data on *E. crista-galli* report or suggest bird pollination, either by hummingbirds (Knuth, 1906; Schrottky, 1908; Werth, 1915; Pickens, 1931; Ali, 1932; Gottsberger et al., 1984) or by hummingbirds and passerine birds (Toledo, 1974; I. Baker & H. G. Baker, 1982; H. G. Baker & I. Baker, 1983, 1990). In particular, orioles have been frequently recorded as visitors and pollinators of *Erythrina* species from the Americas (Feinsinger et al., 1979; Morton, 1979; Toledo & Hernández, 1979). In contrast, Hymenopterans have been rarely mentioned as possible visitors for the genus (Toledo & Hernández, 1979) or for *E. crista-galli* (Ali, 1932; Faegri & van der Pijl, 1971; Duncan in Raven, 1974; Parodi, 1978). Monkeys have been reported elsewhere as visitors in a few New World *Erythrina* taxa. Jaeger (1961) saw howling monkeys on *Erythrina* species from Paraguay, and Janson et al. (1981) indicated that flowers of two species from Peruvian forests were an important food source for monkeys.

Studies on flowers and their animal visitors have led to the assumption that there are coevolutionary relationships between nectar traits and pollinator type. Several authors have discussed nectar volume and concentration of flowers attracting different guilds of pollinators. In general, they found that hummingbird and honeyeater flowers present large amounts of dilute nectar, especially relative to nectars of bee flowers (e.g., Baker, 1975; Pyke & Waser, 1981, and references therein; Cruden et al., 1983; Opler, 1983). Nevertheless, and under laboratory conditions, hummingbirds, honeyeaters, and sunbirds given a choice of sugar solutions have been found to prefer the highest sugar concentrations offered at an equal volume presentation (Hainsworth & Wolf, 1976; Stiles, 1976; Tamm & Gass, 1986; Mitchell & Paton, 1990). On the other hand, studies on the sugar composition of nectar showed that hummingbird flowers produce a sucrose-dominated nectar while those associated with passerine birds produce nectar dominated by glucose and fructose (H. G. Baker & I. Baker, 1983, 1990). Sugar preferences of hummingbirds have been examined in previous studies, which have found that they preferred sucrose solutions instead of equivalent monosaccharide solutions (Stiles, 1976; Martínez del Río, 1990a; Stromberg & Johnsen, 1990). Physiological studies on New World nectarivorous passerine birds have shown a correlation between the sucrose aversion with a relative enzymatic lack of sucrase activity (Martínez del Río et al., 1988, 1989; Martínez del Río, 1990b). However, recent studies on Old World passerine birds have shown that they possess high efficiency in sucrose absorption, and Old World counterparts do not reject sucrose in favor of hexose sugars (Downs & Perrin, 1996; Lotz & Nicolson, 1996; Downs, 1997a, b).

In addition to nectar traits, red flowers can be largely considered as bird-adapted because insects have little or no sensibility to red (e.g., Proctor et al., 1996; Vogel, 1996). However, recent literature suggests that red flowers are not invisible to bees as previously thought (Chittka & Waser, 1997). In particular, for Papilionoideae a conspicuous UV reflectance/absorbance patterning was demonstrated for mellitophilous species but not for ornithophilous ones (Kay, 1987). Because flowers of *E. crista-galli* lack UV patterns and showed little or no UV reflectance (Kay, 1987), red flowers of this species should be seen not only by hummingbirds but also by carpenter bees, the most frequent flower visitor.

The two woody subfamilies Caesalpinioideae and Mimosoideae and primitive woody tribes of the Papilionoideae have mostly maintained self-incompatibility, whereas self-incompatibility has been lost on numerous occasions in herbaceous legumes (Kalin Arroyo, 1981). It is possible that its loss occurred as a consequence of the peculiarities of tripping in papilionoid flowers, because this mechanism permits the maintenance of high levels of outcrossing in many self-compatible legumes (Kalin Arroyo, 1981). Nevertheless, the level of outcrossing will be primarily correlated with the size of flower display, enhancing the possibilities for geitonogamous selfing. In large plants such as trees, much selfing will result from inter-flower transference of pollen.

Previous data on some species indicate that both self-compatibility and self-incompatibility are present in *Erythrina* (cf. Kalin Arroyo, 1981; Neill, 1988). According to Fryxell (1957), *E. crista-galli* is self-incompatible, but later reports (Raven, 1974; Neill, 1988), confirmed herein, indicated that it is self-compatible. According to Cruden and Lyon (1989), facultatively xenogamous species are self-compatible, adapted for cross-pollination, presenting a single flower type, with delayed autogamy, and most show high fruit and seed set. Facultative xenogamy is considered a mixed mating system in

which the level of pollinator activity is the primary determinant of the balance between self- and cross-pollination (Cruden & Lyon, 1989). Facultative xenogamy has been correlated with a moderately high pollen/ovule ratio (i.e., ca. 700; Cruden, 1977). Although *E. crista-galli* presented a pollen/ovule ratio (> 57,000) that coincides with Cruden's (1977) class for obligate xenogamy, we considered this species as facultatively xenogamous because it is self-compatible, the flowers are adapted for cross-pollination, and both pollinator behavior and inflorescence size affect selfing (i.e., mainly geitonogamy). Empirical evidence suggests that numerous plant species have evolved a mixed reproductive strategy (Richards, 1997). An evolutionarily stable strategy can develop for mixed mating, because selfing as well as outcrossing can confer fitness benefits onto offspring (Holsinger, 1991).

Pollinators preferentially visit large inflorescences because they provide a larger signal to attract them and/or offer greater pollen and nectar rewards (e.g., Stephenson, 1979; Augspurger, 1980). When a plant displays numerous flowers simultaneously, geitonogamy may occur if the pollinator moves between flowers. Although plant attractiveness to pollinators often increases with the number of flowers open at one time, display size also bears mating costs to the plant. Aspects of floral design and display that mitigate the mating costs of autogamy but that promote the benefits of enhanced pollinator attraction may be widespread, given that most animal-pollinated plants display several to many flowers each day (Harder & Barrett, 1996). Some deductions on the *E. crista-galli* reproductive strategy can be made when fruit set as well as fruit and seed parameters from hand- and natural-pollinations are compared, particularly when these results are related to inflorescence size, to the number of simultaneously open flowers per tree, and to the foraging behavior of the flower visitors. The possibility of regularly occurring natural selfing would explain the low natural fruit set and low seed germinability observed in *E. crista-galli*. That quantitative traits from fruits and seeds obtained by natural pollination are more similar to those from selfed flowers than from crossed ones can be related to significant pollen flow within a tree mediated by the flower visitors. Results indicate that *E. crista-galli* shows reproductive plasticity, which allows this species to reproduce successfully despite variation in the quality and quantity of the pollinator guild.

Cladistic analyses of *Erythrina* based on morphological and cpDNA characters suggest that *E. crista-galli* has a basal position and lies sister to the remaining species within the genus (Bruneau, 1996, 1997). Within the primarily entomophilous tribe Phaseoleae, there is some agreement that *Erythrina* is bird-pollinated (Raven, 1974, 1977; Bruneau, 1997). Most *Erythrina* species in the Americas are pollinated by hummingbirds, although perching birds are also common visitors and pollinators of several taxa (Neill, 1987; Bruneau, 1997). In an article on the coevolution of birds with *Erythrina* (Bruneau, 1997), *E. crista-galli* was included within a basal clade with *E. fusca* Lour. and *E. falcata* Benth. with this clade characterized as passerine/hummingbird-pollinated. However, for *E. crista-galli*, we observed not only birds, but also bees as significant visitors and pollinators.

Conclusions

Erythrina crista-galli, like the other species in the genus (Neill, 1988), is self-compatible, showing low fecundity and high rates of flower and fruit abortion. Its showy red flowers produce hexose-dominant nectar and are assiduously visited primarily by hymenopterans (carpenter bees and honeybees) and also by hummingbirds (four species), all of which can be assumed as pollinators. In natural populations, several traits related to pollinator behavior, e.g., high number of simultaneously open flowers per tree, high nectar production per flower, would favor pollen flow within trees. If this is true, a reproductive consequence such as inbreeding depression could occur. An outcome of this situation might be the low quality of selfed seeds compared to seeds derived from outcrossed flowers.

Knowledge of directional changes in pollination and breeding systems can provide valuable clues for reconstructing phylogenies (Kalin Arroyo, 1981). As the genus *Erythrina* seems to be wholly self-compatible (Neill, 1988), information on shifts in pollinators may be valuable. This highly derived genus was previously considered to be ornithophilous within the primarily entomophilous Phaseoleae (Doyle & Doyle, 1993; Bruneau et al., 1995). Cladistic analyses in *Erythrina* included *E. crista-galli* in the basal clade (Bruneau, 1997). However, our data indicate that flowers were primarily visited by bees. This may indicate that the pollinators for this basal clade have departed from entomophily, typical of Phaseoleae, to ornithophily, more typical of *Erythrina*. Further studies on the reproductive biology and frequency of visitors are needed on the other members of this basal *Erythrina* clade for confirmation of this. Our results suggest that the pollination biology of the *Erythrina* species is complex, that the species have a wide array of visitors,

and that more detailed field studies are needed to clarify their evolution.

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THE LONG-PROBOSCID FLY POLLINATION SYSTEM IN SOUTHERN AFRICA[1]

Peter Goldblatt[2] *and John C. Manning*[3]

ABSTRACT

Some 14 species of long-proboscid flies (Diptera) in two families, Nemestrinidae and Tabanidae, with elongated mouth parts are known to pollinate species in several plant families, most importantly Geraniaceae, Iridaceae, and Orchidaceae, across southern Africa. Long-proboscid fly pollination appears to comprise three discrete guilds of flies in the genera *Prosoeca*, *Moegistorhynchus*, and *Stenobasipteron* (Nemestrinidae) and *Philoliche* (Tabanidae). Flies in the three guilds are on the wing at different times of the year and pollinate different suites of plant species, sometimes with different floral characteristics. The three pollinator guilds operate for the most part in different parts of the subcontinent. Where there is geographical overlap, the periods of activity differ. Plants pollinated by long-proboscid flies have flowers with an elongate, cylindrical floral tube, mostly 35–60 cm long, a perianth of specific colors and marking, a floral reward of nectar, and lack floral fragrance. Pollen is not eaten by these flies, and anthers and pollen are often cryptically colored. Flowers are usually zygomorphic and bilabiate, and the petal or tepal lobes have characteristically shaped nectar guides. With few exceptions, the flowers offer ample nectar of sugar concentration mainly in the 20–30% range. Several orchids and one *Pelargonium* using long-proboscid flies for pollination offer no nectar, and pollination is accomplished through floral mimicry and deception. In long-proboscid fly pollination systems, placement of pollen on the insect's body is highly specific, and there are at least six mutually exclusive sites of pollen deposition on an insect's body. When two or more long-proboscid-fly-pollinated plant species co-occur, each typically utilizes a different pollen deposition site. This suggests that pollen contamination is detrimental to reproductive success and that differential pollen deposition sites are important for plants pollinated by long-proboscid flies. Since these flies are the sole or main pollinators of at least 120 plant species and the inferred pollinators of at least 80 more species in southern Africa, they must be considered keystone species in the ecosystems where they occur.

Key words: co-evolution, floral ecology, long-proboscid flies, pollination.

A close association between the form and color of flowers and pollination by a particular pollinator is well known. Convergence in floral morphology among species that rely on the same pollinator class led to the recognition of floral syndromes (e.g., Vogel, 1954; Faegri & van der Pijl, 1979). Moreover, species with morphologically similar flowers that share the same pollinator species constitute a particular pollination guild, an extension of the term describing a group of species that exploit the same class of resources in a similar way (Root, 1967). Likewise, insect species using a particular group of plants as a food resource in a similar way may also be regarded as a guild. A guild is thus a functional unit independent of taxonomic considerations, as are floral syndromes. Although a number of pollination syndromes have been identified in the southern African flora (Vogel, 1954), relatively few guilds have been described. The most striking of those that have been documented is the association between the satyrid butterfly, *Aeropetes* (*Meneris*) *tulbaghia*, and late-summer-flowering species with large, bright red blossoms (Johnson & Bond, 1994). Others include the association of several plant species with magenta to violet-colored flowers and the fly *Prosoeca peringueyi* (Manning & Goldblatt, 1996), the suite of plant species with cream to pale pink flowers blooming in autumn that depend on *P. longipennis* for their pollination (Manning & Goldblatt, 1995), and the *Moegistorhynchus longirostris* guild of the west coast of South Africa.

Pollination by long-proboscid flies is a relatively unusual phenomenon, first recorded in southern Africa by Marloth (1908) and later described in somewhat more detail by Vogel (1954). In their review of insect pollination in the Cape Flora of South Africa, Whitehead et al. (1987) were the first to really recognize long-proboscid fly pollination as a unique pollination system, although very little was then known about either the flies or what plant species

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they pollinated. Thus, long-proboscid fly pollination has only been regarded as a class of pollination system comparable with that of the major pollinator groups or syndromes, bird, bat, bee, butterfly, and muscid/carrion fly, since the late 1980s. Muscid/carrion fly pollination, also called myophily, is classically associated with actinomorphic, bowl- or salver-shaped flowers of pale or dull colors, readily accessible nectar, and well exposed sex organs (Faegri & van der Pijl, 1979). Pollination by Diptera is, in fact, diverse and cannot be usefully regarded as a single pollination syndrome. Sapromyophily is already distinguished from general myophily because the flies in the syndrome have lapping mouth parts, are attracted by unpleasant odors of decay or fermentation, and are associated with flowers with dull colors, often with mottled pigmentation, and hairy or frilled petals or tepals. Long-proboscid fly pollination (or rhinomyophily, viz. Rebelo et al., 1985) may readily be distinguished from classical myophily and its specialized derivative, sapromyophily, and differs in all criteria enumerated by Faegri and van der Pijl for this myophily. Long-proboscid fly flowers are typically zygomorphic, normally have an elongate floral tube, and have bright coloration. The sex organs are presented in a wide range of orientations, sometimes concealed within or above the mouth of the tube, or are elongate and held distant from the source of the nectar reward in a unilateral, arcuate (adaxial) or declinate (abaxial) disposition.

Although pollination by long-proboscid flies has been described from various parts of the world, the system as we define it here is restricted to the Himalayan Region (Fletcher & Son, 1931; Dierl, 1968) and southern Africa, where the system has received a fair amount of attention since 1990 (e.g., Goldblatt et al., 1995; Manning & Goldblatt, 1996, 1997; Johnson & Steiner, 1995, 1997). So-called long-proboscid fly pollination described in the literature, for example, by Grant and Grant (1965) in California, refers to bombyliid flies with probosces less than 15 mm long and differs from the pollination system we regard as long-proboscid fly pollination. Here we review long-proboscid fly pollination, report additional examples of pollination by long-proboscid flies, and assess the importance of the system in the southern African flora relative to other pollination systems.

Review of Long-Proboscid Fly Pollination

long-proboscid flies

Definition and morphological characteristics. We define long-proboscid flies here as those insects that have mouth parts at least 15 mm long and a body length of more than 15 mm. Fifteen species in two families, Nemestrinidae and Tabanidae, are known to have mouth parts this long, 14 of them restricted to the southern African region, Lesotho, Namibia, South Africa, and Swaziland (Fig. 1, Table 1), and one to the Himalayas (Dierl, 1968). Adult morphs of these flies depend largely or exclusively on floral nectar for their nutrition and are avid foragers of nectar-rich flowers (female Tabanidae also require a blood meal). Their visits to the flowers of some plants result in the passive accumulation of pollen or pollinaria as they brush against anthers, and in turn, the passive transference of pollen or pollinaria to stigmas during visits to other flowers of the same species. Most other Nemestrinidae and Tabanidae have substantially shorter mouth parts and, although they also feed on nectar and pollinate plants, they are not known to be the only pollinator(s) of any plants. Instead they share pollen resources with other insect taxa including long-tongued bees, Lepidoptera, hopliine beetles, and bee flies (Bombyliidae) (Goldblatt et al., 1995, 1998b, in prep.). The tabanid, *Philoliche aethiopica*, and the acrocerid flies, *Psilodera* spp. (Goldblatt et al., 1997; Potgieter et al., 1999), have mouthparts of intermediate length, mostly 12–15 mm long, and they are provisionally excluded from consideration here: their shorter mouthparts prevent them from foraging effectively on flowers of plant species that have exclusively long-proboscid fly pollinators.

Long-proboscid flies are large-bodied insects, typically measuring 15–24 mm from the tip of the abdomen to the base of the proboscis. Mouth parts are as long as, or often substantially longer than, the insect's body, the most extreme example being *Moegistorhynchus longirostris*—individuals along the Cape west coast have been recorded with probosces up to 100 mm long (Fig. 2). Foraging behavior is similar in all species, irrespective of family or genus, and although flies have been described as hovering while foraging (Struck, 1997), this is not the usual pattern. Our observations show that flies firmly grasp tepal or petal lobes or other floral organs as they forage for nectar and while doing so they continue to vibrate their wings rapidly (Goldblatt et al., 1995; Goldblatt & Manning, 1999) (Fig. 3A–D).

Foraging patterns vary, but our observations show that long-proboscid flies are seldom flower constant. While flies sometimes forage for a time on a particular floral form and may visit a particular species more frequently than any other, more often their foraging appears to be random, and foraging

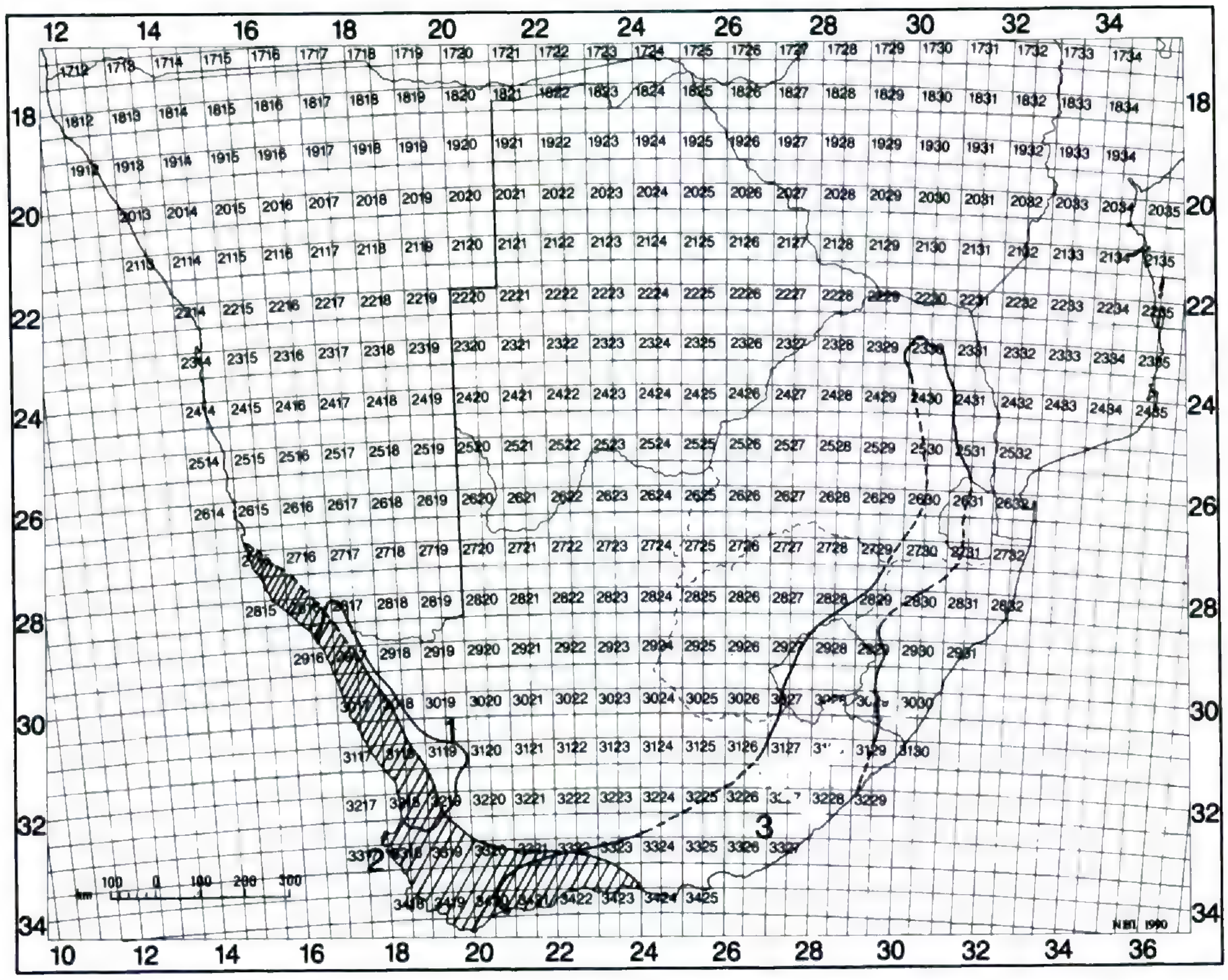

Figure 1. Southern Africa, showing the geographical extent of each of the three long-proboscid fly pollination guilds. —1. The *Prosoeca peringueyi* guild. —2. The *Moegistorhynchus–Philoliche* guild (shaded). —3. The *Prosoeca ganglbaueri* guild. The ranges of guilds 1 and 2 correspond almost exactly to the southern African winter-rainfall zone. Note the limited geographic overlap that does not coincide temporarily.

bouts may include flowers of several different species, some of which even have different shapes, sizes, and colors. This emphasizes that long-proboscid flies can and do forage on a wide range of flowers. Long-tubed flowers, however, are the only ones that offer a secure reward, and because of their size, a significantly larger reward, and one that is not available to most other nectarivorous insects.

Zoogeography. The southern African long-proboscid flies have variable geographic ranges (Table 1). All but one of the species are, however, restricted to one or the other of the two major climatic and biotic zones of southern Africa, the winter-rainfall zone in the southwest, and the summer-rainfall zone, which covers the rest of the subcontinent (Fig. 1). *Prosoeca ganglbaueri* has the widest range and extends from the Northern Province of South Africa through eastern southern Africa to the Kleinswartberg in the south (at the interior edge of the winter-rainfall zone), a distance of over 1500 km. In contrast, and despite pollination research in the area, *P. rubicunda* is known from one specimen from the southwestern Cape, while *P. nitidula* is restricted to the Cape Peninsula at the extreme southwestern edge of the subcontinent and the center of the winter-rainfall zone. Evidently also rare, *Moegistorhynchus* sp. is known from two high-mountain sites in the southwestern Cape.

PLANT SPECIES

Plants that depend on long-proboscid flies for their pollination comprise a varied group taxonomically and morphologically (Table 2). They range from seasonal perennials, mostly geophytes with corms, bulbs, or tubers (Amaryllidaceae, Iridaceae, Orchidaceae, some Geraniaceae), to shrubs (Ericaceae, some Geraniaceae, Lamiaceae, Scrophulariaceae). No annuals or trees have so far been found with this pollination system.

Floral characteristics. Flowers of most species have in common a long floral tube (Figs. 4, 5) (we use the term here to include a corolla or perianth tube, tepal spurs of orchids, as well as the receptacular tube of *Pelargonium*), usually exceeding 20 mm, and usually produce ample nectar. Notable exceptions are *Aristea spiralis* (Iridaceae) and species of *Brunsvigia* and *Nerine* (Amaryllidaceae), which

Table 1. Proboscis length, peak months of activity, and geographic ranges of long-proboscid flies in southern Africa. Data are taken from our observations, the literature, and museum collections. So little is known about *Prosoeca rubicunda* that its inclusion in the *Moegistorhynchus–Philoliche* guild is tentative. Taxonomic affiliation: Nemestrinidae—*Moegistorhynchus, Prosoeca, Stenobasipteron;* Tabanidae—*Philoliche.* WRZ = winter-rainfall zone, SRZ = summer-rainfall zone. *Note that we regard *M. braunsii* and *M. perplexus* (each known only from their type collections) as synonyms of *M. longirostris.*

Fly species	Proboscis length range mm (N)	Months on the wing	Geographic range
Prosoeca peringueyi guild			
P. peringueyi	(15–)25–40 (15)	July–Sep.	WRZ: S Namibia to N Western Cape
P. sp. nov.	32–48 (8)	Aug.–Sep.	WRZ: Northern Cape (Calvinia District)
Moegistorhynchus–Philoliche guild			
*M. longirostris**	(35–)42–80 (14)	Sep.–Nov.	WRZ: Western and Northern Cape coast and interior
M. sp. nov.	ca. 21 (1)	Jan.	WRZ: Western Cape
Philoliche gulosa	18–33 (9)	Sep.–Nov.	WRZ: Western Cape, mainly interior
P. rostrata	21–27 (7)	Oct.–Nov.	WRZ: Western Cape, coast and interior
Prosoeca nitidula	18–28 (5)	Oct.–Jan.	WRZ Western Cape (only Cape Peninsula)
P. rubicunda	ca. 21 (1)	Jan.	WRZ: Western Cape (only Caledon District)
Prosoeca ganglbaueri guild			
P. ganglbaueri	(17–)25–42 (12)	Jan.–Apr.	SRZ & WRZ: Northern Province to E Western Cape incl. Lesotho
P. longipennis	38–40 (3)	Mar.–Apr.	WRZ: Southern Cape
P. robusta	20–46 (7)	Feb.–Apr.	SRZ: Mpumalanga
Stenobasipteron wiedmannii	18–30 (5)	Feb.–Apr.	SRZ: Mpumalanga and KwaZulu-Natal

have floral tubes less than 10 mm long. Species with short floral tubes have elongate stamens so that the body of a foraging fly will brush against anthers even when its proboscis much exceeds the length of the tube.

Flower colors fall into two major groups (Goldblatt et al., 1995; Manning & Goldblatt, 1996, 1997). In northern Western Cape Province and Namaqualand flowers are typically intensely dark red to purple or violet with pale nectar guides (Fig. 4). In the rest of southern Africa, however, these colors are rarely associated with long-proboscid fly flowers. Instead, flowers are usually shades of cream to pink, with pink to red nectar guides (Fig. 5). A few species may have pale blue or mauve flowers, but species of *Nivenia* (Iridaceae) are exceptional in having deep blue perianths. Again with few exceptions, the nectar guides consist of longitudinal streaks. Flower form is usually zygomorphic and bilabiate with unilateral, arcuate stamens and style (Figs. 4G–L, 5F, G) or with declinate stamens and style (Figs. 4D–F, 5B–E). A few species of *Erica, Hesperantha, Ixia, Nivenia, Romulea,* and one of *Lapeirousia* have actinomorphic flowers (Figs. 4A–C, 5A).

Anthers and pollen are often unconventionally pigmented and frequently match the color of the perianth, or are simply a dull blue-gray to mauve (Goldblatt et al., 1995). Some *Gladiolus* and *Tritonia* species with cream flowers, however, have dark purple pollen (Goldblatt & Manning, 1999). It has been suggested that unusually colored pollen is an example of crypsis, making the pollen less conspicuous to pollen-collecting insects (Manning & Goldblatt, 1996). In *Pelargonium* species, anthers and pollen are bright red to orange and may contribute to the floral signal to flies (Goldblatt et al., 1995), especially if the petals are weakly marked.

Scent production is rare (Table 2). In the Iridaceae only *Babiana sambucina* has strongly scented flowers, while *B. framesii* often has lightly fragrant flowers. In the Amaryllidaceae, species of *Brunsvigia* typically have a light sweet fragrance.

A particularly notable aspect of long-proboscid fly pollinated plants is the range of stamen orientation and length, which are directly related to the site of deposition of pollen on the body of a fly. Actinomorphic flowers have symmetrically disposed stamens either held close to the mouth of the floral tube, or in *Erica,* within the tube. In *Aristea spiralis* the anthers are held 16–20 mm from the vestigial floral tube. *Nivenia* species either have the anthers held at least 10 mm from the mouth of the tube, or in the distylous *N. argentea* and *N. steno-*

Figure 2. *Moegistorhynchus longirostris*, the fly with the longest proboscis of all southern African long-proboscid flies. The tongue measures between 35 and 100 mm long. Note the scattering of pollen over the dorsal part of the fly's body.

siphon stamens of the pin (long-styled and short-stamened) morph are held just above the apex of the floral tube. In zygomorphic flowers, the stamens are unilateral and either arcuate (arching above the mouth of the tube), as in most Iridaceae, or declinate (arching below the mouth of the tube), as in *Pelargonium* (Fig. 4D–F), *Geissorhiza* (Fig. 5B), and the amaryllids *Brunsvigia* and *Nerine*. In the latter two genera, the filaments are extended forward for 35–40 mm, so that the anthers are held at least this distance from the nectar source. Stamen length is notably variable in *Pelargonium*, species of which have anthers held close to, or up to, 15 mm from the mouth of the floral tube. Pollen deposition on foraging flies is dorsal in species with arcuate stamens and ventral in species with declinate stamens. When anthers are held close to the mouth of the floral tube, pollen deposition is on the frons and/or the base of the proboscis.

Comparative Pollination Ecology of the Three Guilds

Subdivision of the Long-Proboscid Fly Pollination Syndrome

We propose recognizing three separate pollination guilds or systems within the long-proboscid fly pollination strategy. The flies that belong to each system show little or no overlap with those of other systems in plants visited or season on the wing, although there is some overlap in geographic range (Fig. 1). Within each system there are well-defined guilds of plant species that have one or occasionally two fly species as their sole pollinator. A few plant species may be pollinated by different fly species in different parts of their ranges. Thus, *Lapeirousia silenoides* is pollinated only by *Prosoeca peringueyi*, but *L. jacquinii* may be pollinated by *P. peringueyi* or, on the Bokkeveld Plateau, by *Prosoeca* sp. nov. Likewise, *L. fabricii* has been recorded as pollinated by *Moegistorhynchus longirostris* at some sites, by *Philoliche gulosa* at others, and by both flies at one site (Manning & Goldblatt, 1997). The occasional presence of two long-proboscid fly species at a few sites has also been documented by Goldblatt and Manning (1999) both in Western Cape and Mpumalanga Provinces, but this appears to be a relatively infrequent situation.

1. The *Prosoeca peringueyi* guild includes two fly species, *P. peringueyi* and *Prosoeca* sp. nov. (Table 1). With complementary ranges in southern Namibia and Namaqualand to the Olifants River Valley and the Western Karoo in South Africa (Fig. 1), these two flies are active from July to late September. The range is thus restricted to the western half of the southern African winter-rainfall zone and activity to the cooler months of the year. The two flies pollinate separate suites of species with similar floral presentation, but at least the widespread *Babiana framesii*, two varieties of *B. sambucina*, and *Lapeirousia jacquinii* share both flies as their sole pollinators (Goldblatt et al., 1995; Manning & Goldblatt, 1996).

Plants in the guild (Table 2) stand out in having flowers intensely pigmented in shades of magenta, deep purple, or violet, the lower, or all the petal or tepal lobes in the case of actinomorphic flowers, with cream to yellow markings and areas of darker pigmentation (Fig. 4). Tepal lobes are often relatively small, 12–15 mm long (e.g., *Lapeirousia jacquinii*, *L. silenoides*, *Romulea hantamensis*), but some species of *Babiana* belonging to the guild

have relatively large flowers with tepal lobes up to 28 mm long. Anthers and pollen are often shades of mauve to violet or cream.

The floral tube in most species of the *Prosoeca peringueyi* guild is at least 20 mm long, and usually in the 30–45 mm range. All members of the guild flower in the late winter to late spring, late July to mid September, and are geographically restricted to the southern African west coast and near interior (Namaqualand and the northwestern Cape, South Africa, and southwestern Namibia). Some plant species in the guild have somewhat wider ranges than their pollinators and today reproduce sexually only by autogamously produced seed in the parts of their ranges where no pollinating flies occur (e.g., *Lapeirousia jacquinii, Sparaxis metelerkampiae*) (Manning & Goldblatt, 1996; Goldblatt et al., in prep.).

2. The *Moegistorhynchus–Philoliche* guild, named for the most conspicuous fly genera in the system, includes six or seven fly species. The two tabanids, *P. rostrata* and *P. gulosa*, have the widest ranges, collectively extending from southern Namibia to the southeastern Cape (Fig. 1). The three *Moegistorhynchus* species and the one or two *Prosoeca* species of the system have narrower ranges in the southwestern Cape (Table 1). Flies of this system are mostly active from late spring to early summer, mid September to November, but are still on the wing until January locally at higher elevations. Species pollinated by this group of flies mostly have a similar floral presentation, and the particular pollinator depends on geography. The same species may be pollinated by up to three different flies over its entire range. Although the geographical ranges of the *Pr. peringueyi* and *Moegistorhynchus–Philoliche* systems overlap in western southern Africa, they overlap very little in period of activity of fly species and, as far as is known, not at all in plants pollinated.

Plants in the *Moegistorhynchus–Philoliche* guild mostly have flowers in shades of white to cream, usually with pink undertones, or pale to deep pink (Fig. 5). Nectar guides are deep pink or red, occasionally with a white streak in the center of each dark mark. On the southern African west coast, flowers often have relatively large tepal lobes, 30–50 mm long (e.g., *Gladiolus angustus, G. undulatus, Pelargonium longicaule*), but *Babiana tubulosa, Lapeirousia anceps*, and *P. appendiculatum* have fairly small, inconspicuous tepal or petal lobes, up to 15 mm long. The longest floral tubes (60–110 mm long) are found in west coast populations of *G. angustus, L. anceps*, and *P. appendiculatum*, and these species are associated exclusively with a single pollinator, *Moegistorhynchus longirostris*. There, this fly has a proboscis at least 65 mm long, whereas in central Namaqualand, to the north, it has a proboscis only 37–45 mm long. Populations of *G. angustus* and *L. anceps* elsewhere have shorter tubes and are pollinated by *Philoliche* species, which have correspondingly shorter probosces.

Several plant species from the Roggeveld and Hantam areas of the western Karoo including *Babiana spathacea, Disa karooica*, and *Romulea syringodeoflora* (white-flowered race), as well as several species of *Pelargonium*, all of which have cream to white flowers with red markings and elongate floral tubes, present a problem in the context of long-proboscid fly pollination. Although they bloom from October to early December, when *Philoliche gulosa* and *P. rostrata* are on the wing, and have flowers that appear to be adapted for pollination by long-proboscid flies, no long-proboscid species have been captured or recorded in this area. Can the legitimate pollinator(s), whether one of these *Philoliche* species or another fly species, be extinct locally, as suggested by S. D. Johnson (pers. comm.)?

Too little is known about the Western Cape species, *Prosoeca rubicunda*, to confidently place it in one of the long-proboscid fly guilds, but its apparent period of activity, January, suggests that it may be a local extension of the *Moegistorhynchus–Philoliche* guild, which is centered in Western Cape Province. Several plant species from the Caledon–Bredasdorp area of the winter-rainfall zone that flower in the summer, from November to January, including *Nivenia concinna, N. stokoei*, and *Tritoniopsis flexuosa* (Iridaceae) and *Pelargonium caucalifolium* (Geraniaceae), have no known pollinator although they have flowers that conform closely to the long-proboscid fly type.

3. The *Prosoeca ganglbaueri* guild, named for the most widespread and common fly species in the system, operates in the southern summer and autumn, from January to April, and largely in eastern southern Africa in areas of summer precipitation (Fig. 1), but also in the eastern half of the winter-rainfall zone that receives appreciable summer precipitation. As treated here, the *P. ganglbaueri* guild includes four fly species (Table 1). While *P. ganglbaueri* extends from Northern Province southward to the Kleinswartberg Mountains in Western Cape Province, *P. longipennis* is restricted to the southern Cape coastal belt. Because of the opposed seasonality there is virtually no overlap in plant species with the *P. peringueyi* and *Moegistorhynchus–Philoliche* systems. The ranges of the flies *Prosoeca longipennis* and *P. ganglbaueri*, in the south of its

Figure 3. Long-proboscid flies foraging on their flowers. —A. *Prosoeca peringueyi* hovering above a cluster of *Lapeirousia silenoides* flowers. —B. *Moegistorhynchus longirostris* about to forage on a flower of *Lapeirousia anceps*.

range, overlap the eastern half of the range of flies belonging to the *Moegistorhynchus–Philoliche* guild, but the months of activity of the flies in the two systems differ.

Guilds of plant species pollinated by *Prosoeca ganglbaueri, P. longipennis,* and *P. robusta* overlap to some extent. For example, populations of *Tritoniopsis revoluta* occur within the ranges of *P. ganglbaueri* and *P. longipennis*; *Disa amoena* pollinaria have been found on both *P. ganglbaueri* and *Pr. robusta,* and both flies have been captured while foraging on *Gladiolus calcaratus* and *Watsonia wilmsii.* Likewise, *P. ganglbaueri* and *P. longipennis* have been observed and captured pollinating *Pelargonium dipetalum* in different parts of its range. No overlap has so far been encountered in the species pollinated by *Stenobasipteron wiedmannii,* and further investigation may show this fly represents a guild separate from the *P. ganglbaueri* guild of flies.

Flowers pollinated by flies in the *Prosoeca ganglbaueri* guild are usually pink with dark pink to red markings, but *Zaluzianskya microsiphon* has cream petals, pink on the reverse, and *Gladiolus calcaratus* has white flowers. *Nivenia stenosiphon* is unusual in having actinomorphic, uniformly deep blue flowers, at least to the human eye. Thus, in general flowers pollinated by flies of the *P. ganglbaueri* guild resemble those pollinated by flies of the *Moegistorhynchus–Philoliche* guild. Species pollinated by *P. longipennis,* a fly restricted to the southern Cape coastal belt, have flowers with relatively small petal or tepal lobes, mostly ca. 10 mm long, and a corolla or perianth that ranges from cream to pale pink or salmon, usually with darker pink or red on the lower (*Gladiolus, Tritoniopsis*), or upper (*Pelargonium*), or all the lobes (*Cyrtanthus leptosiphon*) (Manning & Goldblatt, 1995). Flowers pollinated by *Stenobasipteron wiedmannii* are shades of pale blue, mauve, or pink (Goldblatt & Manning, 1999; Potgieter et al., 1999) and usually have small tepal or petal lobes, but species pollinated by *P. ganglbaueri* and *P. robusta* often have large lobes.

Floral Reward—Nectar

Nectar volume. Nectar production is usually ample, and quantities mostly range from 1.1 to 5 μl per flower in a standing crop (unbagged flowers) (Table 3). Large-flowered species like *Gladiolus angustus* and *G. undulatus* may produce up to 10 μl of nectar per bloom. *Aristea spiralis* is unusual in having flowers that offer less than 0.5 μl of nectar. *Disa draconis, D. harveiana,* and *D. oreophila, Pelargonium sericifolium,* and *Hesperantha scopulosa* produce no nectar (Table 3) and evidently depend for their pollination on deceit, their flowers closely resembling those of other members of their respective pollination guilds (Goldblatt et al., 1995; Johnson & Steiner, 1995, 1997; Manning & Goldblatt, 1997).

Nectar sugar chemistry. In species of the Iridaceae, Lamiaceae, and Orchidaceae, nectar is sucrose-rich to sucrose-dominant (ratio of sucrose to hexose sugars greater than 1—see Table 3), but nectar in most species of *Pelargonium* examined is hexose-rich or hexose-dominant. Nectar sugar concentrations are mostly in the 20–30% sucrose equivalent range, but may be as low as 19% (*Romulea hantamensis*) or as high as 38% (*P. incrassatum*) (Table 3). Two species of *Hesperantha, H. grandiflora* and *H. galpinii,* also produce nectar of unusually low sugar concentration, less than 18% sucrose equivalents.

Pollen Placement

Pollen placement on the body of a fly appears to be an important consideration in long-proboscid fly pollination systems. At all sites that we have investigated, the number of plant species utilizing a particular long-proboscid fly for their pollination appears to be closely correlated with the number of pollen deposition sites (Manning & Goldblatt, 1996, 1997). Typically the frons and base of the proboscis, the dorsal part of the head and thorax, and the ventral part of the thorax and abdomen are used by different plant species for pollen deposition (Fig. 6). In the case of some Orchidaceae, pollinaria are usually deposited near the base of the proboscis (Johnson & Steiner, 1995, 1997). The need for specific sites for pollen or pollinarium deposition is presumably related to the behavior of long-proboscid flies, which are not flower constant. Instead, they visit flowers of different species in an apparently random pattern on foraging bouts (Goldblatt et al., 1995; Goldblatt & Manning, 1999). The deposition of more than one pollen species at the

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—C. *Prosoeca* sp. nov. about to insert its proboscis into the long perianth tube of *Lapeirousia oreogena.* —D. *Philoliche rostrata* about to forage on *Tritonia flabellifolia*; note the heavy deposit of purple *Tritonia* pollen on the dorsal part of the thorax.

Table 2. Floral characters of plants pollinated by long-tongued flies arranged by guild. Abbreviations: b = blue with light throat; cr = pale cream with red markings; p = pale pink or deep pink with red or purple nectar guides; v = vivid (red, purple, or violet shades with contrasting pale nectar guides); wh = white. Scent indicated by − = absent; + = present; ++ = present and strong. For tube length we give only the functional length, not total length—in *B. curviscapa*, *B. dregei*, and *B. framesii*, all acaulescent species, the lower part of the tube is closed and only serves to raise the flower above the foliage. Additional species inferred on the basis of floral presentation to belong to particular guilds, but with no insect visits recorded, are listed in parentheses. References, column eight, are as follows: 1 = Goldblatt et al. (1995) and Manning & Goldblatt (1996); 2 = Manning & Goldblatt (1997); 3 = Goldblatt & Manning (1999); 4 = Goldblatt et al. (1999); 5 = Johnson & Steiner (1995); 6 = Johnson & Steiner (1997); 7 = Struck (1997); 8 = Manning & Goldblatt (1995); 9 = Vogel (1954); 10 = Goldblatt & Bernhardt (1990); 11 = Potgieter et al. (1999); 12 = Goldblatt & Manning (new data; for methodology see Goldblatt et al. (1998a), Goldblatt & Manning (1999)).

Species	Flower: Sym-metry	Flower: Color	Flower: Scent	Pollinator	Mouth part mm	Floral tube mm	Reference
Prosoeca peringueyi pollination system							
Geraniaceae							
Pelargonium							
echinatum Curtis	z	wh	−	*Pr. peringueyi*	n/a	n/a	7
incrassatum (Andr.) Sims	z	v	−	*Pr. peringueyi*	28–33	30–40	1
magenteum van der Walt	z	v	−	*Pr. peringueyi*	30–35	33–39	1
sericifolium van der Walt	z	v	−	*Pr. peringueyi*	35–40	35–60	1
Iridaceae							
Babiana							
curviscapa G. J. Lewis	z	v	−	*Pr. peringueyi*	25–28	26–36	1, 12
dregei Baker	z	v	−	*Pr. peringueyi*	25–28	30–35	1
ecklonii Klatt	z	v	−	*Pr. peringueyi*	27–32	40–50	12
flabellifolia G. J. Lewis	z	v	−	*Pr.* sp. nov.	40–45	40–65	1
framesii L. Bolus	z	v	+	*Pr.* sp. nov. and	40–45	45–50	1
				Pr. peringueyi	30–35	45–50	1
geniculata G. J. Lewis	z	v	−	*Pr. peringueyi*	30–35	35–45	1
pubescens G. J. Lewis	z	v	−	*Pr. peringueyi*	32–35	44–50	1
sambucina							
var. *longibracteata* G. J. Lewis	z	v	++	*Pr.* sp. nov.	40–45	40–50	12
var. *unguiculata* G. J. Lewis	z	v	+	*Pr. peringueyi*	40–45	40–50	12
Hesperantha							
latifolia (Klatt) M. P. de Vos	z	v	−	*Pr. peringueyi*	15–25	20–25	1
Lapeirousia							
dolomitica subsp.							
dolomitica Dinter	z	v	+	*Pr. peringueyi*	33–35	35–40	1
jacquinii N. E. Br.	z	v	−	*Pr.* sp. nov. and	33–40	33–35	1
				Pr. peringueyi	30–35	33–35	1
oreogena Schltr.	a	v	−	*Pr.* sp. nov.	40–45	53–70	1
pyramidalis subsp. *regalis* Goldblatt & J. C. Manning	z	v	−	*Pr. peringueyi*	32–34	40–47	1
silenoides (Jacq.) Ker Gawl.	z	v	−	*Pr. peringueyi*	35–40	43–55	1
violacea Goldblatt	z	v	−	*Pr. peringueyi*	32–35	34–40	1
Romulea							
hantamensis (Diels) Goldblatt	a	v	−	*Pr.* sp. nov.	40–45	50–70	1
Sparaxis							
metelerkampiae (L. Bolus) Goldblatt & J. C. Manning	z	v	−	*Pr. peringueyi*	30–35	40–45	1

Table 2. Continued.

Species	Flower: Symmetry	Flower: Color	Flower: Scent	Pollinator	Mouth part mm	Floral tube mm	Reference
[Inferred members of the guild—Geraniaceae: *Pelargonium cortusifolium* L'Hér., *P. crassicaule* L'Hér. Iridaceae: *Lapeirousia dolomitica* subsp. *lewisiana* (B. Nord.) Goldblatt, *Geissorhiza kamiesmontana* Goldblatt, *Hesperantha oligantha* Diels, *H. purpurea* Goldblatt, *Romulea kamiesensis* M. P. de Vos, *Sparaxis roxburghii* (Baker) Goldblatt, *Tritonia marlothii* M. P. de Vos, *Xenoscapa uliginosa* Goldblatt & J. C. Manning. Scrophulariaceae: *Jamesbrittenia fruticosa* (Benth.) Hilliard]							
				Moegistorhynchus–*Philoliche* pollination system			
Campanulaceae							
Lobelia							
coronopifolia L. (pink form)	a	p	–	*Ph. gulosa*	18–20	ca. 20	12
Geraniaceae							
Pelargonium							
articulatum (Cav.) Willd.	z	cr	–	*Ph. rostrata*	n/a	50–75	7
barklyi Scott Elliot	z	cr	–	*Ph. rostrata*	n/a	n/a	7
elongatum (Cav.) Salisb. (also as *P. zonale*)	z	cr	–	*Ph. gulosa* and	22–34	24–32	1, 2, 12
				Ph. rostrata	22–31	24–32	12
laevigatum (L.f.) Willd.	z	p	–	*Ph. gulosa*	n/a	n/a	7
				and *Ph. rostrata*	n/a	n/a	7
longicaule Jacq.	z	p	–	*M. longirostris*	65–72	40–90	2
				Ph. rostrata	ca. 35	55–66	5
myrrhifolium (L.) L'Her.	z	cr	–	*Ph. rostrata*	23–27	18–23	5
oxyphyllum DC.	z	p	–	*Ph. gulosa*	18–20	20–25	12
patulum Jacq.	z	p	–	*Ph. rostrata*	25–27	15–20	2, 7
peltatum (L.) L'Her.	z	p	–	*Ph. rostrata*	27–31	22–33	7, 12
				and *Ph. gulosa*	17–19	22–33	12
praemorsum (Andr.) Dietr.	z	cr	–	*M. longirostris*	27–40	35–40	2
Iridaceae							
Aristea							
spiralis (L.f.) Ker Gawl.	z	b/w	–	*Ph. gulosa*	16–18	ca. 1.5	12
Babiana							
tubulosa (Burm. f.) Ker Gawl.	z	w	–	*M. longirostris*	70–95	65–72	2
Geissorhiza							
bonaspei Goldblatt	z	p	–	*Ph. rostrata*	22–24	22–30	12
				and *Pr. nitidula*	18–24	22–30	12
exscapa (Thunb.) Goldblatt	z	p	–	*M. longirostris*	65–70	65–80	2
confusa Goldblatt	z	p	–	*Ph. rostrata*	25–27	35–40	12
				and *Ph. gulosa*	22–24	35–40	12
Gladiolus							
angustus L.	z	p	–	*M. longirostris*	67–70	70–110	2
carneus D. Delaroche	z	p	–	*Ph. rostrata*	22–24	32–37	3
				and/or *Pr. nitidula*	18–24	32–37	3
floribundus Jacq.	z	cr	–	*Ph. rostrata*	30–34	45–60	3
				and *Ph. gulosa*	17–18	45–60	3
monticola G. J. Lewis ex Goldblatt & J. C. Manning	z	p	–	*Ph. rostrata*,	25–27	22–30	3
				Pr. nitidula	18–20	22–30	3
rhodanthus J. C. Manning & Goldblatt	z	p	–	*M.* sp. nov.	18–20	23–27	3
undulatus Jacq.	z	cr	–	*Ph. rostrata*	25–27	52–75	2, 3
vigilans Barnard	z	p	–	*Ph. rostrata*	23–25	35–40	3
virgatus Goldblatt & J.C. Manning	z	p	–	*Ph. rostrata*	18–20	23–27	3

Table 2. Continued.

Species	Flower: Sym-metry	Color	Scent	Pollinator	Mouth part mm	Floral tube mm	Reference
Ixia							
bellendenii R. C. Foster	a	p	−	*Ph. gulosa*	18–22	30–32	4
paniculata D. Delaroche	a	cr	−	*M. longirostris*	67–70	65–75	2, 6
				and *Ph. rostrata*	n/a		12
paucifolia G. J. Lewis	a	cr	−	*Ph. gulosa*	18–22	26–28	4
				and *Ph. rostrata*	ca. 16	16–18	4
Lapeirousia							
anceps (L.f.) Ker Gawl.	z	p	−	*M. longirostris*	52–63	65–76	1, 2
				and *Ph. gulosa*	29–34		2
fabricii (D. Delaroche) Ker Gawl.	z	cr/p	−	*M. longirostris*	42–70	38–65	1, 2
				and *Ph. gulosa*	29–34	n/a	2
Tritonia							
crispa (L. f.) Ker Gawl.	z	p	−	*M. longirostris*	42–46	40–48	2
				and *Ph. gulosa*	29–34	40–48	2
flabellifolia (D. Delaroche) G. J. Lewis	z	p	−	*Ph. rostrata*	27–31	45–55	12
				and *Ph. gulosa*	17–18	45–55	12
pallida Ker Gawl.	z	p	−	*Ph. rostrata*	30–34	45–55	12
Watsonia							
borbonica (Pourr.) Goldblatt	z	p	+	*Ph. rostrata*,	19–22	14–18	12
				Pr. nitidula, anthophorine and megachilid bees, *Ph. lateralis*	18–19.5	14–18	12
paucifolia Goldblatt	z	p	−	*M.* sp. nov.	18–20	22–30	12
Orchidaceae							
Disa							
draconis (L. f.) Sw.	z	cr	−	*M. longirostris*,	ca. 57	ca. 48	6
				Ph. rostrata	ca. 35	57–72	6
harveiana Lindl.	z	pb	−	*Ph. rostrata*	23–27	32–38	6

[Inferred members of the guild—Ericaceae: *Erica aristata* Andr., *E. embothriifolia* Salisb., *E. jasminiflora* Salisb., *E. junonia* Bolus, *E. praecox* Klotzsch, *E. shannoniana* Andr. Geraniaceae: *Pelargonium alchemilloides* (L.) L'Her., *P. appendiculatum* (L.f.) Willd., *P. articulatum* (Cav.) Willd., *P. denticulatum* Jacq., *P. moniliforme* E. Mey. ex Harv., *P. oblongatum* Harv., *P. punctatum* (Andr.) Willd., *P. radiatum* (Andrews) Pers., *P. stipulaceum* (L.f.) Willd., *P. suburbanum* Cliff., *P. tetragonum* (L.f.) L'Hér. Iridaceae: *Babiana brachystachys* G. J. Lewis, *B. spathacea* (L.f.) Ker Gawl., *Geissorhiza callista* Goldblatt, *G. longifolia* (G. J. Lewis) Goldblatt, *G. schinzii* (Baker) Goldblatt, *G. stenosiphon* Goldblatt, *G. tenella* Goldblatt, *Gladiolus leptosiphon* F. Bolus, *G. variegatus* (G. J. Lewis) Goldblatt & J. C. Manning, *Ixia fucata* Ker Gawl., *I. splendida* G. Lewis, *Lapeirousia macrospatha* Bak., *L. simulans* Goldblatt & J. C. Manning, *L. verecunda* Goldblatt, *Nivenia argentea* Goldblatt, *Romulea albiflora* Goldblatt & J. C. Manning, *Thereianthus elongatus* (Schltr.) G. J. Lewis, *Tritonia bakeri* Klatt, *T. cooperi* (Baker) Klatt, *T. lancea* (Thunb.) N. E. Br., *T. tugwelliae* L. Bolus, *Watsonia dubia* Klatt, *W. strictiflora* Ker Gawl. Orchidaceae: *Disa karooica* Johnson & Linder, *D. longicornu* L.f., *D. marlothii* Bolus, *D. porrecta* Sw., *D. salteri* G. J. Lewis.]

Prosoeca ganglbaueri–Pr. robusta pollination system

Amaryllidaceae							
Brunsvigia							
grandiflora Lindl.	z	p	+	*Pr. ganglbaurii*	31–42	>10	12
gregaria R. A. Dyer	z	p	+	*Pr. ganglbaurii*	31–42	>10	12
striata (Jacq.) Aiton	z	p	+	*Pr. ganglbaurii*	34–36	>10	12

Table 2. Continued.

Species	Flower			Pollinator	Mouth part mm	Floral tube mm	Reference
	Sym-metry	Color	Scent				
Nerine							
cf. *angustifolia* (Baker) Watson	z	p	–	*Pr. robusta*	ca. 33	>5	12
bowdenii S. Watson	z	p	–	*Pr. ganglbaueri*	27–30	ca. 5	12
Geraniaceae							
Pelargonium							
carneum Jacq.	z	p	–	*Pr. ganglbaueri*	34–36	55–60	12
				Pr. longipennis	38–40	55–60	8
dipetalum L'Her.	z	cr/p	–	*Pr. longipennis*	38–40	ca. 60	8
gracillimum Fourc.	z	p	–	*Pr. ganglbaurii*	34–36	50–60	12
pinnatum (L.) L'Her.	z	cr/p	–	*Pr. longipennis*	38–40	50–60	8
Iridaceae							
Gladiolus							
bilineatus G. Lewis	z	p	–	*Pr. longipennis*	38–40	50–60	8
calcaratus G. J. Lewis	z	w	–	*Pr. robusta*	20–23	28–40	3
engysiphon G. Lewis	z	cr	–	*Pr. longipennis*	38–40	52–60	3
macneilii Oberm.	z	p	–	*St. wiedmannii*	23–29	40–45	3
microcarpus G. J. Lewis	z	p	–	*Pr. ganglbaueri*	27–30	35–40	3
mortonius Herbert	z	p	–	*Pr. ganglbaueri*	31–42	36–52	3
oppositiflorus J. D. Hook.	z	p	–	*Pr. ganglbaueri*	28–32	40–50	12
varius F. Bolus	z	p	–	*Pr. ganglbaueri*	33–35	45–55	3
				and *Pr. robusta*	20–30	45–55	12
Hesperantha							
brevicaulis (Baker) G. J. Lewis	a	p	–	*St. wiedmannii*	18–30	25–37	12
grandiflora G. J. Lewis	z	p	–	*Pr. ganglbaueri*	23–25	33–36	12
scopulosa Hilliard	a	p	–	*Pr. ganglbaueri*	32–35	32–40	12
cf. *woodii* R. C. Foster	a	p	–	*Pr. ganglbaueri*	27–31	35–38	12
Nivenia							
stenosiphon Goldblatt	a	b	–	*Pr. ganglbaueri*	23–25	32–38	10
Tritoniopsis							
revoluta (Burm.f.) Goldblatt	z	p	–	*Pr. ganglbaueri*	23–25	ca. 40	12
Watsonia							
wilmsii N. E. Br.	z	p	–	*Pr. ganglbaueri*	33–35	40–45	12
				and *Pr. robusta*	36–45	40–45	12
Lamiaceae							
Orthosiphon							
tubiformis R. Good	z	p	–	*St. wiedmannii*	23–29	28–35	12
Plectranthus							
ambiguus (Bol.) Codd	z	b	–	*St. sp.*	20–33	22–29	11
ecklonii Benth.	z	ma	–	*St. wiedmannii*	16–21	24–26	11, 12
hilliardii Codd	z	b	–	*St. sp.*	21–29	22–29	11
Orchidaceae							
Disa							
oreophila Bolus	z	p	–	*Pr. ganglbaueri*	19.6(2.3)	20.6(1.1)	5
amoena Linder	z	p	–	*Pr. ganglbaueri*	33–35	25–30	12
				and *Pr. robusta*	20–30	25–30	12
Brownleea							
coerulea Harv. ex Lindl.	z	m	–	*St. wiedmannii*	ca. 23	20–24	12
macroceras Sond.	z	p	–	*Pr. ganglbaueri*	19.6(2.3)	38.9(6.4)	5

Table 2. Continued.

Species	Flower: Symmetry	Flower: Color	Flower: Scent	Pollinator	Mouth part mm	Floral tube mm	Reference
Scrophulariaceae							
Zaluzianskya							
microsiphon (Kuntze) K. Schum.	z	w	–	*Pr. ganglbaueri*	33–35	30–42	12

[Inferred members of the guild—Acanthaceae: *Ecbolium glabratum* Vollesen, *Isoglossa cooperi* C. B. Cl., *Rhinacanthus gracilis* Klotzsch, *Salpinctium natalense* (C. B. Cl.) T. J. Edwards, *Siphonoglossa linifolia* (Lindau) C. B. Cl. Amaryllidaceae: *Brunsvigia* spp., *Cyrtanthus leptosiphon* Snij., *C.* sp. nov., *Nerine filamentosa* W. F. Barker, *N. krigei* W. F. Barker. Caryophyllaceae: *Dianthus basuticus* Burtt Davy. Ericaceae: *Erica cerinthoides* L. (pink form). Geraniaceae: *Pelargonium acraeum* R. A. Dyer, *P. caucalifolium* Jacq. subsp. *caucalifolium*, *P. ionidiflorum* (Eckl. & Zeyh.) Steud., *P. transvaalense* Knuth. Iridaceae: *Geissorhiza fourcadei* (L. Bolus) G. Lewis, *Gladiolus cataractarum* Oberm., *G. saxatilis* Goldblatt & J. C. Manning, *G. scabridus* Goldblatt & J. C. Manning, *Hesperantha coccinea* (Backh. & Harv.) Goldblatt & J. C. Manning, *H. curvula* Hilliard & Burtt, *H. hutchingsii* Hilliard & Burtt, *H. huttonii* (Baker) Hilliard & Burtt, *H. pulchra* Baker, *Nivenia concinna* N.E. Br., *N. stokoei* L. Guthrie, *Radinosiphon leptostachya* (Baker) N. E. Br., *Tritoniopsis flexuosa* (Thunb.) G. Lewis, *Watsonia occulta* N. E. Br. Lamiaceae: *Syncolostemon densiflorus* Benth., *S. macranthus* (Garke) Ashby, *S. rotundifolius* E. Mey., *Thorncroftia longiflora* N. E. Br., *T. media* Codd, *T. succulenta* (Dyer & Bruce) L. E. Codd. Orchidaceae: *Disa rhodantha* Schltr., *D. saxicola* Schltr., *Satyrium hallackii* Bolus. NOTE—*Hesperantha pubinervia* Hilliard & Burtt may belong here, but although it does have an elongate perianth tube, the tube itself is extremely narrow, accommodating only the style, and it does not produce nectar.

Anecdotal observations of long-proboscid flies visiting flowers but no claims for pollination or evidence of pollen transfer to stigmas.

Ericaceae. *Erica junonia* Bolus (?*Ph. rostrata*—E. G. H. Oliver, pers. comm.).

Scrophulariaceae. *Jamesbrittenia fruticosa* (Benth.) Hilliard (*Pr. peringueyi*—Museum record); *Zaluzianskya microsiphon* (*Pr. peringueyi*—Museum record).

Geraniaceae. *Pelargonium alchemilloides* (L.) L'Her. (*Ph. rostrata*—Vogel, 1954); *P. denticulatum* (unidentified fly—McDonald in Struck, 1997); *P. suburbanum* (*M. longirostris*—Johnson & Steiner, 1997); *P. tetragonum* (unidentified fly—van Jaarsveld in Struck, 1997).

same site on the insect's body would frequently result in insect visits failing to accomplish pollination as a result of stigma clogging by foreign pollen.

In the *Prosoeca peringueyi* pollination guild pollen deposition on the ventral head or thorax is effected by *Pelargonium* species, on the frons or dorsal thorax by *Lapeirousia* species (normally one or two species of a genus is present at any site), and on the dorsal thorax by *Babiana* species (also normally only one species present at any site) (Manning & Goldblatt, 1996). Pollen of *Hesperantha latifolia*, occasionally part of the guild using *P. peringueyi* as pollinator, is deposited on the lateral and upper ventral thorax. In *P. incrassatum* and *P. sericifolium*, which are sympatric at some sites in Namaqualand and are both pollinated by *P. peringueyi*, pollen contamination is avoided by placement of their respective pollen on the ventral head or ventral thorax of *P. peringueyi*, the result of shorter or longer filaments in these two species.

This pattern is repeated in the *Moegistorhynchus longirostris–Philoliche* pollination guild (Manning & Goldblatt, 1997). Pollen deposition on the distal ventral thorax is effected by *Pelargonium* spp. (either *P. praemorsum* or *P. longicaule*) or *Geissorhiza* spp. (either *G. confusa* or *G. exscapa*) and on the proximal ventral thorax or lower head by *P. tabulare*. Deposition on the frons is effected by *Lapeirousia anceps*, the dorsal head or thorax by *Tritonia crispa* at some sites, and by *Gladiolus undulatus*, *G. angustus*, or *L. fabricii* at others. *Ixia paniculata* is unusual in having short stamens held within the mouth of the tube, and its pollen is deposited on the frons around the base of the proboscis. Species of Orchidaceae that have stalked pollinaria are probably not directly involved in competition for pollen deposition, but the number of species of Orchidaceae at any site is usually limited to one.

Pollen deposition in the *Prosoeca ganglbaueri* guild follows this general pattern. For example, anthers of *Brunsvigia*, *Nerine*, and *Pelargonium* spp. brush different parts of the ventral head, thorax, or abdomen, depending on stamen length, while pollen of *Gladiolus* and *Watsonia* species is deposited on the dorsal thorax. Orchid pollinaria are placed near the base of the proboscis. Pollen placement

by *Hesperantha* species, which mostly have symmetrically disposed, divergent anthers, is less well defined but is always on the lateral or ventral parts of a fly's body. Pollen of the important long-proboscid fly plant, *Zaluzianskya microsiphon*, is deposited on the ventral head as the anthers are held at the abaxial side of the mouth of the floral tube.

Evolution of the System

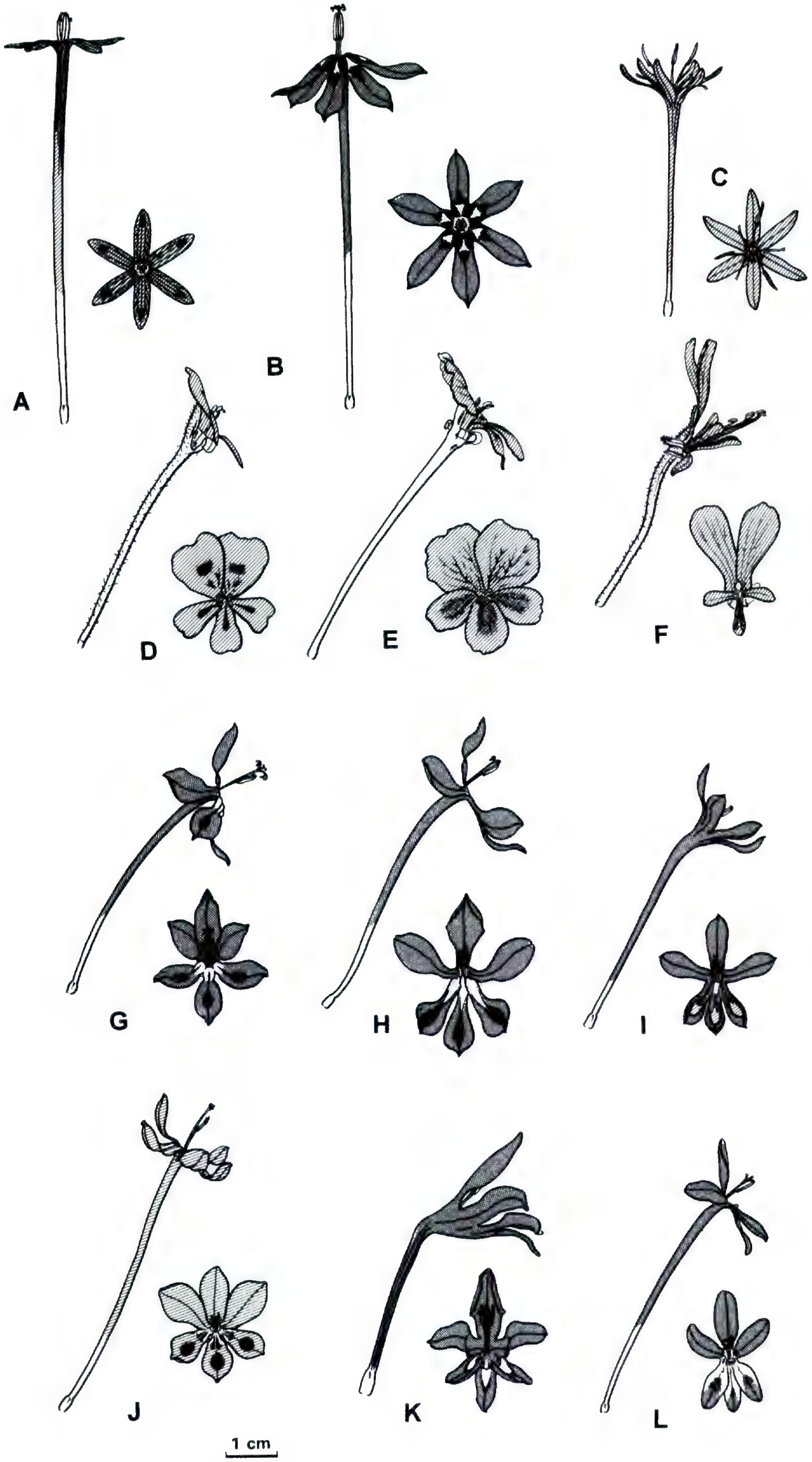
Associated with butterfly pollination by Vogel (1954) in his keystone work on pollination systems in the flora of southern Africa, long-proboscid fly pollination appears to have no direct connection with that strategy. Butterfly and long-proboscid fly pollination have some similarities, notably including plant species with odorless, long-tubed flowers. The two systems are, however, independent of one another, and no species of either group of insects share the flowers of any plant species. Moreover, butterfly pollination in southern Africa (excluding the highly specialized *Aeropetes* butterfly system—Johnson & Bond, 1994) is frequently a part of generalist systems that use other insects, including bees, hopliine scarab beetles, and settling moths (Goldblatt et al., 1995). In the Iridaceae, phylogenetic studies in *Gladiolus*, *Lapeirousia*, and *Sparaxis* show that long-proboscid fly flowers are nearly always most closely related to ancestors pollinated by long-tongued bees (Apidae) (Goldblatt et al., 1995, in prep.; Goldblatt & Manning, 1999). Species of *Gladiolus* pollinated by long-proboscid flies fall in taxonomic sections where long-proboscid bee pollination is ancestral and in which butterfly pollination may not occur. In the only section of *Gladiolus* in which butterfly pollination (by the specialist pollinator, *Aeropetes*) is significant, section *Blandus*, butterfly flowers most likely evolved from long-proboscid-fly-pollinated ancestors (Goldblatt & Manning, 1998). Pollination by the *Aeropetes* butterfly does not occur in *Babiana* or *Lapeirousia*, genera in which long-proboscid fly pollination is especially common, nor in *Ixia* or *Sparaxis*, or *Tritonia*.

In most families in which long-proboscid fly pollination occurs, a shift from bee to long-proboscid fly pollination appears to be relatively straightforward, involving shifts in perianth color and marking pattern, elongation of the floral tube, and loss of floral odor if present in the ancestor. For example, in most Iridaceae, long-proboscid bee flowers are zygomorphic and have a funnel-shaped perianth tube of moderate length, with the upper flared portion about as long as the cylindric basal half. Unilateral arcuate stamens are held above the mouth of the perianth tube where they readily brush against the body of a large bee as it forces its upper body into the upper part of the floral tube while extending its proboscis into the lower part of the tube. The perianth may be almost any color, but is not often red or cream, and the flowers are frequently scented (Goldblatt et al., 1998a). Long-proboscid fly flowers of the *Moegistorhynchus–Philoliche* and the *Prosoeca ganglbaueri* pollination systems merely require a shift in flower coloring toward the pale pink to cream end of the color spectrum, the acquisition of darker, linear nectar guides, and an elongation of the narrow part of the floral tube to exclude nectar feeders with mouth parts less than 15 mm long. Nectar quality, especially sugar constituents, is much the same as is found in long-proboscid bee flowers, but nectar volume is typically much greater and sometimes more dilute. For example, flowers of bee-pollinated species of *Gladiolus* have nectar volumes between 0.5 and 4 μl compared with 1.8 to over 10 μl in long-proboscid fly pollinated species (Goldblatt et al., 1998a; Goldblatt & Manning, 1999).

It is noteworthy that fly diversity is higher in the geographically much smaller winter-rainfall zone. Three fly species range over all of eastern southern Africa, whereas there are 10 fly species in the two long-proboscid fly guilds in the winter-rainfall zone. Some of these flies are relatively widespread, but *Moegistorhynchus* sp. nov. and *Prosoeca* sp. nov., *P. rubicunda*, and *P. nitidula* have very narrow ranges and moreover, except for *P.* sp. nov., they appear to be rare, at least as far as one can judge from the few specimens known.

Terminology

There is unavoidable confusion when comparing long-proboscid fly and long-proboscid (or long-tongued) bee pollination. Long-proboscid fly pollination, according to our definition, includes flies with probosces in excess of 15 mm and usually much more. Few long-proboscid bees have probosces longer than 12 mm. Moreover, acrocerids, tabanids, and nemestrinids with probosces 10–15 mm long are frequently referred to as long-proboscid flies to contrast them with short-tongue flies that lap fluid. Rebelo et al. (1985) coined the term rhinomyophily, which is useful but not favored by many biologists who prefer more direct terms (e.g., bird pollination vs. ornithophily, etc.). Struck (1997) favored the term hoverfly pollination, but that has the disadvantage of misrepresenting the typical behavior of the flies, which grasp floral organs whenever possible while foraging although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they do hover while inserting the proboscis into a floral tube. We have no solution to offer and suggest *long-proboscid fly pollination* for the syndrome involving flies with probosces usually exceeding 15 mm, and *short-proboscid fly pollination* for the syndrome involving flies with true sucking mouth parts usually less than 15 mm long. Flies with lapping mouth parts would be excluded from this definition. The confusion between long-proboscid fly and long-proboscid bee syndromes as regards length of mouth parts seems unavoidable.

Nectar Considerations

Although most data available for flowers pollinated by Diptera suggest that flies favor nectar with hexose sugars predominant (Baker & Baker, 1983, 1990), these authors did not distinguish between flies with short, lapping mouth parts and those with long, sucking mouth parts. Recent studies, however, show that nectar of plants with flowers adapted for pollination by long-proboscid flies is often sucrose-rich to sucrose-dominant (Goldblatt & Bernhardt, 1990; Goldblatt et al., 1995; Manning & Goldblatt, 1996, 1997; Goldblatt & Manning, 1999). The broader survey here in general confirms this observation, but the data indicate that this may simply be the result of the taxa involved. Most Iridaceae belonging to subfamily Ixioideae (which includes most species pollinated by long-proboscid flies) have sucrose-rich nectar as do all species of the family with flowers adapted for pollination by bees. Significantly, nectar of *Aristea spiralis,* the only species of Iridaceae with long-proboscid fly flowers and not a member of Ixioideae, has hexose-rich nectar. It also produces nectar from perigonal nectaries (Goldblatt & Manning, 1996), unlike species of Ixioideae, which have septal nectaries (Goldblatt, 1990, 1991). Flowers of some other families with long-proboscid fly flowers (e.g., Lamiaceae, Orchidaceae) also have sucrose-rich nectar. However, Geraniaceae does not follow this pattern; species of *Pelargonium* that have been examined (Manning & Goldblatt, 1996, 1997) have, with the exception of *P. cortusifolium,* hexose-rich to hexose-dominant nectar. Among long-proboscid fly flowers then, nectar sugar chemistry may simply be a reflection of systematic affiliation or nectary type, and not the result of selection by long-proboscid flies for a preferred type of nectar. This reflects the conclusions of van Wyk (1993) and van Wyk et al. (1993) that the nectar sugar characteristics of flowers are highly conserved and that nectar sugar patterns often reflect taxonomic affinities rather than pollination systems.

Nectar concentration is relatively constant, although it seldom rises above 32% sucrose equivalents and seldom dips below 20%. The two species of *Hesperantha* in the *Prosoeca ganglbaurii* guild are exceptional in having nectar concentrations below 20%. Concentrations above 32% may make the nectar too viscous to be easily sucked into the proboscis.

A Co-Evolved System

As suggested by Feinsinger (1983) for sphinx moths, it seems that long-proboscid flies and their flowers have probably evolved through reciprocal selection. Frequent visits from long-proboscid flies select for long-tubed flowers, which in turn select for longer-proboscid flies capable of reaching the nectar within the tubes. This pattern fits the Red Queen effect (van Valen, 1973; Futuyma, 1979), in which species may coevolve indefinitely, some becoming extinct in the process, or else arrive at a static, nonevolving equilibrium. Evolution within this system may not, however, be all that simple. Population densities of long-proboscid flies appear to be highly erratic (Goldblatt et al., 1995; Goldblatt & Manning, 1999), a situation comparable to that for sphinx moths, as noted by Gregory (1963–1964). But whereas shorter-proboscid moths visit these flowers, taking advantage of nectar welling up in the tubes, there do not seem to be alternative shorter-proboscid insects available for most long-proboscid fly flowers to use this resource when their primary pollinators are not available. Instead, those long-proboscid fly flowers that are self-incompatible simply fail to reproduce or reproduce poorly in certain seasons at certain sites (Goldblatt & Manning, 1999).

Fail-safe mechanisms for self-pollination appear to be quite common among species pollinated by long-proboscid flies. We know now that of the ten species of *Lapeirousia* with flowers adapted for pollination by long-proboscid flies, four at least are self-compatible and autogamous and two are self-

←

Figure 4. Representative examples of flower form in species pollinated by the long-proboscid flies of the *Prosoeca peringueyi* pollination guild. —A. *Romulea hantamensis.* —B. *Lapeirousia oreogena.* —C. *Hesperantha oligantha.* —D. *Pelargonium ericifolium.* —E. *P. magenteum.* —F. *P. crassicaule.* —G. *Lapeirousia pyramidalis.* —H. *L. dolomitica.* —I. *L. violacea.* —J. *L. silenoides.* —K. *Sparaxis metelerkampiae.* —L. *Tritonia marlothii.*

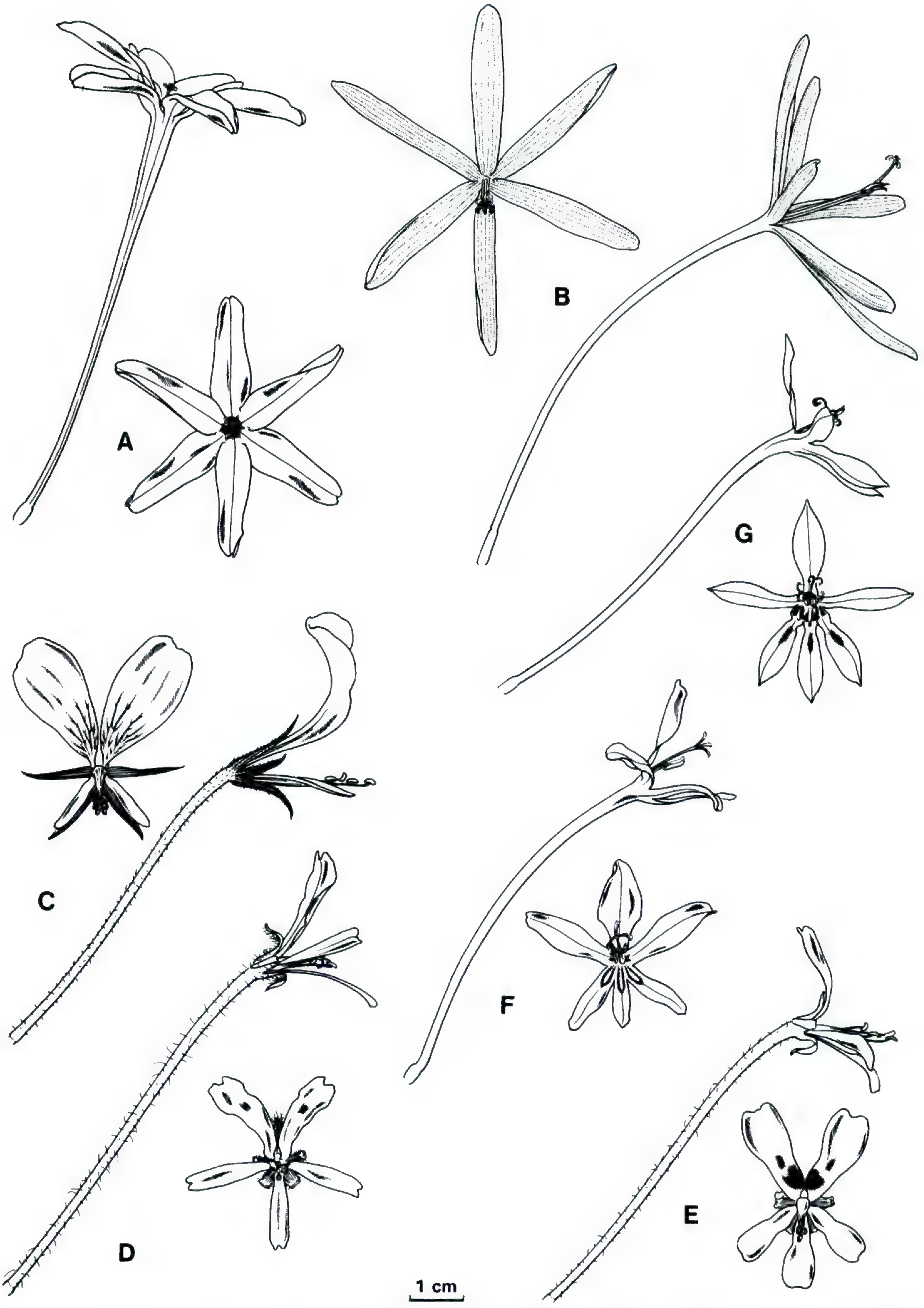

Figure 5. Representative examples of flower form in species pollinated by the long-proboscid flies *Moegistorhynchus longirostris* and *Philoliche gulosa*. —A. *Ixia paniculata*. —B. *Geissorhiza exscapa*. —C. *Pelargonium longicaule*. —D. *P. appendiculatum*. —E. *P. moniliforme*. —F. *Babiana tubulosa*. —G. *Lapeirousia anceps*.

Table 3. Nectar sugars in species of plants pollinated by long-proboscid flies, including data for some species inferred to belong to this pollinator class. Nectar analyses were provided by B.-E. van Wyk. Species are grouped by pollination system. Standard deviation in parentheses after mean nectar sugar concentration is given only for samples greater than 2. References, column eight, are as follows: 1 = Goldblatt et al. (1995) and Manning & Goldblatt (1996); 2 = Manning & Goldblatt (1997); 3 = Goldblatt & Manning (1999); 4 = Goldblatt et al. (1999); 5 = Johnson & Steiner (1997a); 6 = Johnson & Steiner (1997b); 7 = Struck (1997); 8 = Manning & Goldblatt (1995); 9 = Vogel (1954); 10 = Goldblatt & Bernhardt (1990); 11 = Potgieter et al. (1999); 12 = Goldblatt & Manning (new data; for methodology see Goldblatt et al. (1998a), Goldblatt & Manning (1999)).

Species	Nectar		Range of sugars %			Sucrose/ F + G (n)	Reference
	Volume µl (n)	Conc.% (SD)	Fru	Glu	Suc		
			Prosoeca peringueyi pollination system				
Geraniaceae							
Pelargonium							
cortusifolium	2.6–3.1 (2)	22–26	16–19	14–19	62–70	1.94 (2)	1
incrassatum	1.5–2.2 (2)	32–38	0	75	25	0.33 (1)	1
magenteum	0.6–1.8 (6)	27.3 (2.89)	38	40	22	0.28 (1)	1
sericifolium			no measurable nectar produced				1
Iridaceae							
Babiana							
curviscapa	2.0–4.4 (5)	28.0 (2.0)	9–12	14–19	58–73	1.90 (4)	1
dregei	3.9–9.6 (5)	23.3 (2.2)	13–15	19–21	64–68	1.94 (2)	1
ecklonii	4.3–8.9 (5)	27.7 (2.0)	5–11	10–18	72–85	3.29 (3)	12
flabellifolia	3.9–9.6 (5)	26.8 (1.6)	3–7	10–14	75–79	4.53 (2)	1
framesii	2.6–6.4 (10)	28.3 (2.2)	7–9	12–14	77–81	3.84 (3)	1
geniculata	3.2–4.8 (5)	29.3 (1.2)	17	21	62	1.63 (1)	1
pubescens	3.2–4.8 (5)	28.0 (1.7)	3–8	9–14	78–88	2.18 (2)	1
sambucina							
var. *longibracteata*	3.9–6.6 (2)	28–32	6–12	10–19	69–84	3.48 (3)	12
Hesperantha							
grandiflora	2.8–4.1 (5)	14.8 (1.0)	—	—	—	—	12
latifolia	0.7–1.1 (10)	23.5 (2.4)	23–29	24–30	41–53	0.94 (3)	1
oligantha	1.1–1.8 (5)	26.4 (3.9)	19–23	24–25	52–57	1.20 (2)	2
woodii	1.2–1.8 (5)	18.1 (0.9)	—	—	—	—	12
Lapeirousia							
dolomitica							
subsp. *dolomitica*	1.4–3.1 (5)	30.4 (2.4)	4–9	12–14	77–84	4.13 (2)	1
jacquinii	1.5–2.3 (6)	27.3 (2.9)	8	17	75	3.00 (1)	1

Table 3. Continued.

Species	Nectar		Range of sugars %			Sucrose/ F + G (n)	Reference
	Volume µl (n)	Conc.% (SD)	Fru	Glu	Suc		
oreogena	2.5–7.3 (10)	25.8 (1.6)	11–17	19–25	58–69	1.90 (4)	1
pyramidalis subsp. *regalis*	2.6–4.8 (10)	28.4 (2.4)	4–21	12–31	48–84	2.45 (6)	1
silenoides	1.7–3.6 (10)	26.5 (1.1)	5–8	18–27	65–77	2.45 (3)	1
violacea	1.4–1.8 (10)	27.0 (2.9)	9–13	15–16	71–76	2.77 (2)	1
Romulea							
hantamensis	3.7–5.2 (2)	19–21	23	27	50	1.00 (1)	1
Sparaxis							
metelerkampiae	1.7–2.2 (6)	28.5 (3.4)	1–12	4–22	70–95	3.23 (4)	1
Tritonia							
marlothii	2.1–3.5 (5)	29.3 (1.5)	13–14	17–18	68–70	2.23 (2)	12
		Moegistorhynchus–Philoliche pollination system					
Ericaceae							
Erica							
junonia	0.5–1 (4)	22–25	—	—	—	—	12
Geraniaceae							
Pelargonium							
appendiculatum	1.5–2.1 (2)	23–27	55	45	0	0 (2)	2
cf. *barklyi*	2.6–3.2 (6)	25.8 (2.8)	70–78	14–15	7–17	0.14 (2)	12
moniliforme	1.3–2.2 (2)	19–21.5	50–51	41–42	6	0.06 (2)	2
longicaule	1.8–3.6 (5)	20.2 (2.6)	48–64	36–40	0	0 (3)	2
myrrhifolium	2.3–3.6 (5)	20.2 (2.6)	—	—	—	—	12
patulum	—	—	45–55	35–43	2–20	0.12 (2)	12
praemorsum	1.3–4.2 (3)	29.3 (0.6)	55	45	0	0 (1)	2

Table 3. Continued.

Species	Nectar		Range of sugars %			Sucrose/ F + G (n)	Reference
	Volume µl (n)	Conc.% (SD)	Fru	Glu	Suc		
Iridaceae							
Aristea							
spiralis	>0.5 (2)	9–11	28–32	35–36	33–36	0.53 (2)	12
Babiana							
brachystachys	5.3–8.2 (5)	23.4 (1.2)	18–19	24–27	54–58	1.28 (2)	2
spathacea	4.3–5.5 (3)	29.3 (1.1)	13–16	16–18	66–71	2.17 (2)	12
tubulosa	4.5–6.2 (10)	24.9 (1.3)	9–15	14–22	63–77	2.57 (3)	2
Geissorhiza							
bonaspei	2.4–4.1 (10)	24.9 (1.4)	14–15	16–19	66–70	2.13 (3)	12
confusa	2.5–4.8 (10)	19.6 (2.2)	17–21	16–18	61–67	1.78 (2)	12
exscapa	2.7–3.5 (10)	22.8 (2.7)	16–17	19	64–65	1.4 (2)	2
tenella	2.1–2.8 (5)	24.4 (2.1)	—	—	—	—	2
Gladiolus							
angustus	3.9–9.6 (5)	27.8 (2.2)	1–2	6–11	87–93	9.00 (2)	2
carneus	1.6–2.4 (2)	29.0–31.0	0–7	9–13	80–91	5.89 (2)	3
floribundus	2.8–3.7 (2)	28.0–29.5	—	—	—	—	3
monticola	2.4–4.2 (10)	33.2 (3.6)	3–5	10–14	81–85	5.12 (3)	3
undulatus	6.6–10.6 (3)	25.3 (2.6)	2–3	12	85–86	5.90 (2)	2
virgatus	2.8–4.2 (4)	29.9 (0.8)	0–5	4–8	87–96	9.71 (3)	3
Ixia							
bellendenii	2.0–2.4 (6)	23.7 (3.3)	14–19	12–22	60–75	1.94 (3)	12
paniculata	3.9–5.7 (8)	26.1 (2.7)	16–19	17–23	58–66	1.66 (3)	2
paucifolia	1.1–1.6 (5)	29.8 (1.8)	17–21	20–25	54–63	1.41 (2)	12
Lapeirousia							
anceps	1.5–2.4 (10)	25.6 (1.7)	6–20	20–26	54–74	1.78 (3)	1, 2
fabricii	1.3–3.4 (10)	32.5 (2.3)	12–21	21–26	53–67	1.15 (4)	1, 2
	2.4–3.1 (10)	26.0 (3.1)	—	—	—	—	
verecunda	0.6–3.0 (10)	26.6 (2.5)	8	18	74	2.85 (1)	1, 2

Table 3. Continued.

Species	Nectar		Range of sugars %			Sucrose/ F + G (n)	Reference
	Volume µl (n)	Conc.% (SD)	Fru	Glu	Suc		
Tritonia							
crispa	2.4–3.2 (10)	26.1 (4.4)	8–12	11–15	73–81	3.40 (3)	2
flabellifolia	2.3–3.4 (3)	23.2 (1.5)	8–16	11–14	70–81	3.17 (3)	12
pallida	1.5–4.2 (5)	27.7 (1.5)	—	—	—	—	12
Orchidaceae							
Disa							
draconis	no measurable nectar produced						6, 12
harveiana	no measurable nectar produced						6
	Prosoeca ganglbaueri–robusta pollination system						
Amaryllidaceae							
Cyrtanthus							
sp. nov.	4.5–6.8 (5)	14.0 (2.92)	—	—	—	—	12
Geraniaceae							
Pelargonium							
carneum	1.7–2.4 (3)	26–32	—	—	—	—	8
dipetalum	1.5–1.7 (5)	28.3 (1.7)	—	—	—	—	8, 12
pinnatum	0.8–2.2 (5)	22.3 (1.5)	—	—	—	—	8, 12
Iridaceae							
Gladiolus							
bilineatus	4.7–5.6 (3)	27.2 (1.4)	4	11	85	5.67 (1)	8
engysiphon	2.8–4.3 (5)	26.8 (1.4)	0	16	84	5.25 (1)	8
macneilii	4.5–5.8 (5)	26.8 (1.9)	—	—	—	—	3
microcarpus	3.8–4.5 (5)	26.0 (1.1)	—	—	—	—	3
mortonius	4.4–5.7 (10)	27.7 (1.6)	5–11	16–19	70–79	2.85 (3)	3
oppositiflorus	6.4–12.8 (6)	27.3 (1.3)	6–8	14–20	74–78	3.17 (2)	12
varius	6.5–8.9 (7)	30.3 (2.4)	2–6	10–18	76–88	4.80 (4)	3
Hesperamtha							
grandiflora	2.8–4.1 (5)	14.8 (1.0)	—	—	—	—	12
scopulosa	no measurable nectar produced						
woodii	1.2–1.8 (5)	18.1 (0.9)	—	—	—	—	12

Table 3. Continued.

Species	Nectar		Range of sugars %			Sucrose/ F + G (n)	Reference
	Volume µl (n)	Conc.% (SD)	Fru	Glu	Suc		
Nivenia							
argentea	1.6–2.8 (6)	26.7 (2.2)	—	—	—	—	
stenosiphon	ca. 2 (3)	22–38 (n/a)	32	30	38	0.62 (1)	12
Radinosiphon							
leptostachya	1.8–2.6 (3)	19.5 (1.3)	—	—	—	—	12
Tritoniopsis							
revoluta	2.3–2.9 (2)	30–34	23	17	60	1.5 (1)	12
Watsonia							
wilmsii	2.8–4.3 (4)	26.3 (0.9)	4–17	16–22	61–89	2.51 (4)	12
Lamiaceae							
Orthosiphon							
tubiformis	2.7–4.1 (10)	24.5 (2.2)	0–1	0–5	94–100	25.20 (3)	12
Orchidaceae							
Brownleea							
coerulea	1.3–1.8 (2)	25–27	10	0–11	79–90	5.45 (2)	12
macroceras	2.8–4.6 (5)	27.5 (3.3)	—	—	—	—	12
Disa							
amoena	no measurable nectar produced						5
Scrophulariaceae							
Zaluzianskya							
microsiphon	2.6–4.3 (5)	32.6 (1.2)	—	—	—	—	12

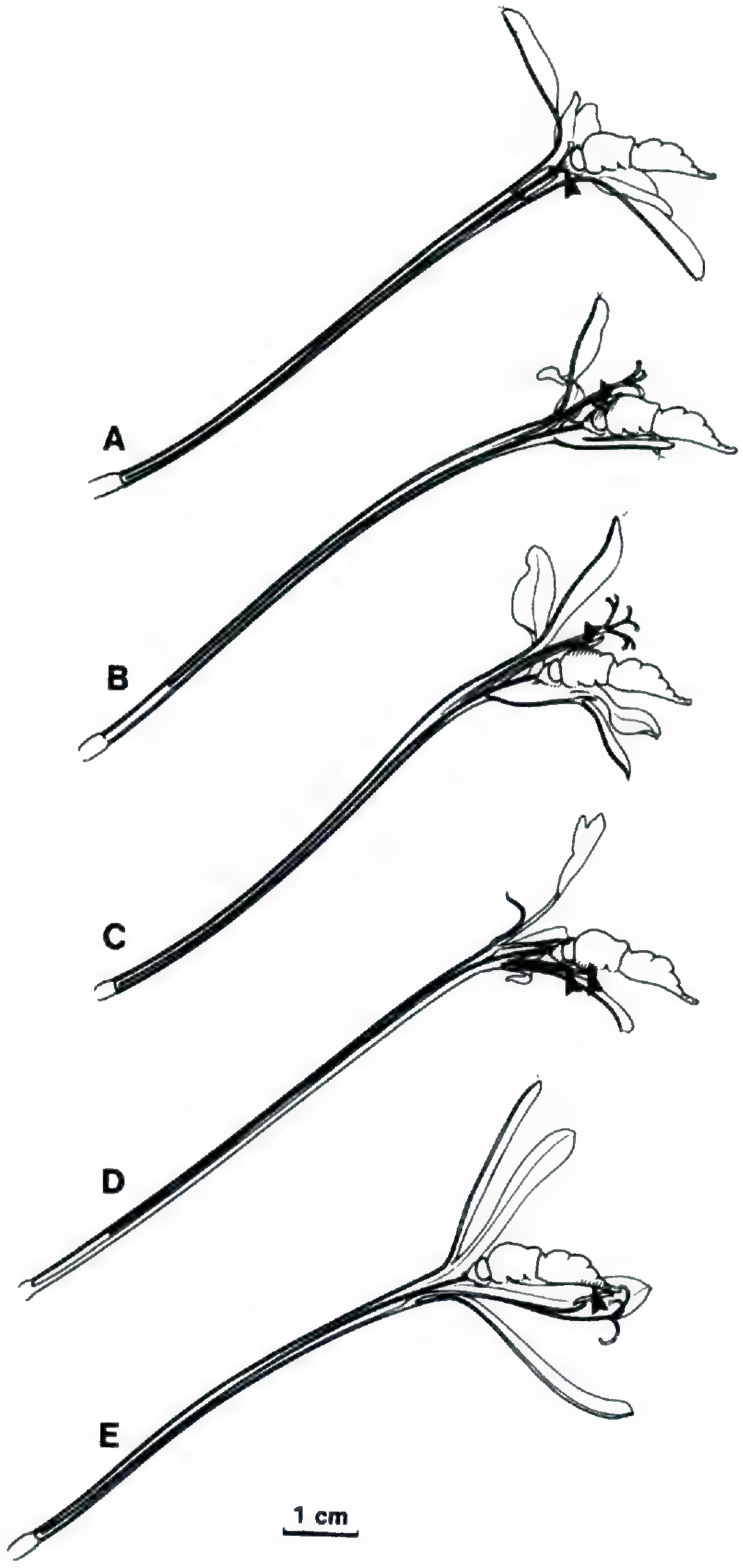

Figure 6. Differential pollen deposition on the body of a fly in a guild of plant species pollinated by *Moegistorhynchus longirostris*. —A. *Ixia paniculata* (frons and proboscis base). —B. *Tritonia crispa* (dorsal thorax). —C. *Lapeirousia fabricii* (dorsal thorax). —D. *Pelargonium appendiculatum* (ventral thorax). —E. *Geissorhiza exscapa* (ventral abdomen).

incompatible (Goldblatt et al., 1995, and unpublished data). At least one species of *Hesperantha* (*H. latifolia*) and the *Ixia* and *Sparaxis* species known to be pollinated by long-proboscid flies are facultatively autogamous, whereas in *Gladiolus* self-incompatibility appears to be frequent (Goldblatt et al., 1998a, in prep.). Some populations of *Gladiolus* species with long-proboscid fly flowers have set no capsules for three years for which we have observations when their pollinators were absent. Although in *Pelargonium* self-compatibility appears to be the rule (Struck, 1997), autogamy cannot normally take place because of mechanical protandry. There is therefore no barrier to geitonogamous pollination in this genus in which inflorescences produce numerous flowers contemporaneously but pollination must be mediated by an external agent.

Proboscis length is typically shorter than the floral tube, a feature that long-proboscid fly pollination shares with sphinx moth pollination. This ensures that a visiting insect must push its body into flowers so that its proboscis will extend as deeply as possible into the tube and reach the nectar reservoir. Orchid genera are one notable exception. In deceptive flowers of *Disa amoena* and the *Disa draconis* complex (Johnson & Steiner, 1997), spur lengths may be shorter than the proboscis length and pollinaria are attached to the upper third of the proboscis. Other exceptions are *Aristea spiralis* and species of *Brunsvigia* and *Nerine* that are pollinated by long-proboscid flies; in these taxa floral tubes are less than 10 mm long. These flies, however, have bodies large enough and probosces long enough that they will consistently brush against anthers and accumulate deposits of pollen on their ventral thoraces and abdomens. Nectar produced by such short-tubed flowers is obviously accessible to a range of other insects, and we have observed effective pollination by bees in *Aristea spiralis*. We have not recorded visits to any other of these short-tubed species by insects other than long-proboscid flies. The considerable distance of the anthers from the mouth of the tube also makes it unlikely that even large bees would normally contact anthers when foraging for nectar in such flowers because their bodies are so much shorter than the filaments.

Long-Proboscid Fly Pollination in the Himalayas

Attention was first drawn to long-proboscid fly pollination in Asia by Fletcher and Son (1931), who briefly described visits to (and inferring pollination of) *Roscoea purpurea* (Zingiberaceae) by the tabanid fly *Chorizoneura longirostris* (proboscis 50–60 mm long). Subsequently, Dierl (1968) described in detail the behavior of this fly in the course of its visits to flowers (presumably foraging for nectar) on *Roscoea*. The Himalayan region is floristically rich and includes several plant species with long-tubed flowers that may also be pollinated by this fly, e.g., other species of *Roscoea* and *Rhododendron* spp. (Ericaceae). This pollination guild awaits more extensive investigation. Involving different fly genera and plant species, the Himalayan system presum-

ably evolved independently of the long-proboscid fly guilds of southern Africa.

Taxonomic Distribution of Long-Proboscid Fly Pollination

According to our survey, at least 83 species have been shown to be pollinated solely by long-proboscid flies. Based on their nearly identical floral presentation, it seems reasonable to infer that at least 90 more species also have this pollination strategy. Even if this figure is grossly underestimated, there seems no doubt that long-proboscid fly pollination is of relatively minor importance in the flora of southern Africa, which comprises over 20,500 species of flowering plants (Goldblatt, 1997). Nevertheless, long-proboscid fly pollination assumes more than marginal importance in at least two families in southern Africa, Geraniaceae and Iridaceae. Precise figures are not available for Geraniaceae, but Struck (1977) has estimated that 25% of the southern African species of *Pelargonium* are pollinated by long-proboscid flies, although the exact definition of the system that he entertained is not clear. In Iridaceae we have more accurate figures. Some 105 of the approximately 1025 species in southern Africa appear to have this pollination system exclusively and one or two more (e.g., *Hesperantha coccinea*—S. D. Johnson, pers. comm.; *Aristea spiralis, Watsonia borbonica*—Table 2) may have a combined long-proboscid fly and other insect pollination. Thus, about 10% of southern African Iridaceae have adopted this pollination strategy. This is substantially more than the estimated 64 species (6.3%) of the southern African Iridaceae that are predicted on the basis of floral morphology to be pollinated by sunbirds (Goldblatt et al., 1999a).

Among the Iridaceae, long-proboscid fly pollination appears to be most well developed in *Lapeirousia,* in which 10 species, or 30% of the total in temperate southern Africa, have flowers pollinated by long-proboscid flies. The system is also well developed in *Gladiolus* (Goldblatt & Manning, 1999; Manning & Goldblatt, 1999), in which 27 species are considered to have flowers adapted for this particular pollination strategy (17% of the southern African species), and long-proboscid fly pollination is inferred for between 10 and 20% of the southern African species of five other large genera, *Babiana, Geissorhiza, Hesperantha, Ixia,* and *Tritonia.*

Long-proboscid fly pollination assumes more modest importance in Ericaceae (Rebelo et al., 1985) and Orchidaceae, although prominent in *Brownleea* and *Disa,* and we have yet to assess its significance in Amaryllidaceae and Lamiaceae. The strategy seems likely in Acanthaceae, but it has yet to be confirmed in that family. In the remaining families in which it occurs, long-proboscid fly pollination is decidedly rare and is evidently confined to just one or a few species. Curiously, evolution of the system seems highly labile in several genera of the Iridaceae and in *Disa* (Orchidaceae). In *Disa* and *Gladiolus* it has evolved repeatedly in different lineages (Johnson et al., 1998; Goldblatt & Manning, 1999), an estimated nine times in the latter genus.

Conservation

Long-proboscid fly pollination poses important concerns for conservation. Plants pollinated by a single insect, or no more than two over their entire range, clearly are at more significant risk than those that are pollinated by several different insects (Bond, 1994). Long-proboscid flies may be regarded as keystone species, for several plant species depend on particular flies for their pollination and sexual reproduction. Conservation of plants with these specialist pollinators must involve conservation of their pollinators, an undertaking fraught with unusual difficulties. Both Nemestrinidae and Tabanidae have complex life cycles. Tabanidae have carnivorous, aquatic larvae that require wetland habitats for their larval development, which may take place some distance from sites of the plant species on which the adults feed. Female tabanids also require a blood meal during their adult phase before egg-laying can proceed. This makes the presence of suitable host mammals essential. Nemestrinidae have an equally complex life history. Although no details of the life cycles of long-proboscid flies are known, all members of the Nemestrinidae so far studied have parasitic larvae, often on locusts. Obviously, large, relatively undisturbed sites with a diversity of habitats are necessary for the completion of the life cycles of nemestrinids and tabanids.

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WOOD ANATOMY OF *MACKINLAYA* AND *APIOPETALUM* (ARALIACEAE) AND ITS SYSTEMATIC IMPLICATIONS[1]

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ABSTRACT

Wood anatomy was examined in both species of *Apiopetalum* (trees endemic to New Caledonia) and in two species of *Mackinlaya* (shrubs from Queensland, Australia), using light and scanning electron microscopy, to investigate their phylogenetic relationships and taxonomic position within Apiales. These genera share several wood features: small intervessel pits, ranging from 3 to 6 μm; both paratracheal and apotracheal (diffuse and diffuse-in-aggregates in *Apiopetalum* only) axial parenchyma; non-septate fibers; rays composed of mostly upright and square cells; and brown deposits in the vessels. Differences in wood structure between the genera appear to be related to habit. The results confirm recent molecular sequence data suggesting that *Apiopetalum* and *Mackinlaya* form a monophyletic group. Evidence from wood anatomy neither validates nor refutes the hypothesis that these two genera are intermediate between Araliaceae and Apiaceae and offers no clear indication of the group's phylogenetic position. Several wood characters (small intervessel pits, thick fiber walls, non-septate fibers) suggest a relationship with *Myodocarpus*, *Delarbrea*, and *Pseudosciadium*. No clear synapomorphies were found to support a sister relationship between *Apiopetalum* and *Mackinlaya* and core Apiaceae as previously suggested by Plunkett, nor between them and other Araliaceae. Helical thickenings on the walls of both ray and axial parenchyma, previously reported only once in *Trigonia* (Trigoniaceae), were observed in one sample of *M. macrosciadea*.

Key words: Apiales, *Apiopetalum*, Araliaceae, *Mackinlaya*, New Caledonia, phylogeny, Queensland, wood anatomy.

The phylogenetic positions of *Apiopetalum* Baill. and *Mackinlaya* F. Muell. within Araliaceae have been difficult to assess using traditional approaches based on morphology. Recent studies using molecular data have provided new insights into their evolutionary relationships, but their exact placement remains unresolved. Five species of *Mackinlaya* (including *Anomopanax*) were recognized by Philipson (1979), extending from Queensland, Australia, through the Solomon Islands, Bismarck Archipelago, New Guinea, and Celebes to the Philippines (see also Philipson, 1951). *Apiopetalum* comprises two species, both endemic to New Caledonia (Lowry, in prep.). *Mackinlaya* species are branched, sympodial shrubs with simple and palmately lobed or palmately compound leaves. *Apiopetalum* species are small trees, to ca. 6 m in height, and have exclusively simple leaves.

In most classification schemes proposed for Araliaceae (Bentham, 1867; Harms, 1894–1897; Viguier, 1906; Hutchinson, 1967; Tseng & Hoo, 1982; Takhtajan, 1987), *Apiopetalum* and *Mackinlaya* have been placed in a separate tribe Mackinlayeae, which has traditionally been distin-

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guished on only a few floral characters, primarily the presence of valvate, clawed petals. While Mackinlayeae have been recognized in nearly all classifications since that of Bentham (1867), this is more a reflection of sparse information on *Apiopetalum* and *Mackinlaya* than any real confidence in their relatedness. We do not consider similarities in the form and position of the petals as sufficient evidence to support the hypothesis that *Apiopetalum* and *Mackinlaya* form a monophyletic assemblage, and additional data are required to clarify their position within Araliaceae.

Apiopetalum and *Mackinlaya* have generally been regarded as most closely related to the well-defined group comprising *Myodocarpus, Delarbrea,* and *Pseudosciadium* (tribe Myodocarpeae), which is centered in New Caledonia (Lowry, 1986a, b). Although the monotypic genus *Pseudosciadium* was included in Mackinlayeae by Harms (1894–1897) because of its valvate, clawed petals, Baillon (1878, 1879) originally suggested that it was most closely related to *Delarbrea* and *Myodocarpus.* Baillon's initial interpretation has been supported by recent systematic studies, and data from the fruit structure (Lowry, 1986a, b) and wood anatomy (Oskolski et al., 1997) indicated that *Myodocarpus, Delarbrea,* and *Pseudosciadium* form a monophyletic assemblage. Results of recent phylogenetic analyses based on *mat*K, *rbc*L, and ITS sequence data (Plunkett, 1994; Plunkett et al., 1996a, b, 1997, unpublished data) further support the hypothesis that these three genera form a distinct, basally branching clade within Apiales. These studies also suggest that *Apiopetalum* and *Mackinlaya* comprise a second, isolated clade within the order.

Apiopetalum and especially *Mackinlaya* resemble many Apiaceae in certain features, such as the presence of clawed petals, inflexed anthers in bud, a bicarpellate gynoecium, and a petiole base that forms a dilated sheath extending around the entire circumference of the stem (the latter two characters occur only in *Mackinlaya*). On this basis, Philipson (1970) and Rodriguez (1957, 1971) regarded *Apiopetalum* and *Mackinlaya* as possible intermediates between Araliaceae and Apiaceae, traditionally regarded as sister groups. In the molecular studies the exact position of the *Apiopetalum–Mackinlaya* clade within Apiales varies depending on the gene sequence and the type of analysis. In some trees, this clade is sister to Apiaceae (comprising subfamilies Apioideae and Saniculoideae, but excluding many genera traditionally placed in Hydrocotyloideae), whereas in other analyses the two genera are sister to the clade comprising the remaining Araliaceae.

Wood anatomical characters can provide useful complementary data for assessing relationships among the genera of Araliaceae, as shown by several previous studies (Oskolski, 1994, 1995, 1996; Oskolski et al., 1997). Until now, no information on the wood anatomy for either *Apiopetalum* or *Mackinlaya* has been available. The present study surveys the wood anatomy of both species of *Apiopetalum* and two of the five species of *Mackinlaya.* The results are examined with regard to hypothesized relationships between these genera, as well as with *Myodocarpus, Delarbrea,* and *Pseudosciadium,* core Araliaceae, and certain woody Apiaceae (*Bupleurum, Heteromorpha, Steganotaenia, Myrrhidendron, Eryngium, Gymnophyton, Asteriscum,* and *Trachymene*).

Materials and Methods

Most wood specimens examined were collected by the authors and G. M. Plunkett; one sample of *Mackinlaya macrosciadea* was provided by B. Hyland (CSIRO Division of Plant Industry, Atherton), and another was taken from a plant at the Sydney Botanic Gardens. Voucher herbarium specimens from New Caledonia are deposited at MO, NOU, and P, and from Australia at QRS and MO. For the following descriptions, in cases where multiple samples of a species were examined and a feature was seen in only a portion of the material, the corresponding collections are indicated in square brackets. Wood samples were taken from trunks in *Apiopetalum* (from a primary branch in *A. velutinum* [3854]), and from basal portions of stems in *Mackinlaya.*

Standard procedures for the study of wood structure were employed to prepare sections and macerations for light-microscopic (LM) studies (Carlquist, 1988). Specimens for scanning electron microscopy (SEM) were prepared according to Exley et al. (1977). Descriptive terminology and measurements follow Carlquist (1988) and the IAWA List of Microscopic Features for Hardwood Identification (IAWA Committee, 1989), except that for the diameter of intervessel pits the vertical dimension was recorded because it is a more constant feature than the horizontal diameter in taxa with opposite and scalariform pitting.

Results

1. *Apiopetalum* (figs. 1–4, 8–12; tables 1, 2).

Material studied. Apiopetalum glabratum Baill.: NEW CALEDONIA, Mé Ori, 830 m, *Lowry 3375*; 850 m, *Lowry 4798. A. velutinum* Baill.: NEW CALEDONIA, Mt. Mou, 1080 m, *Lowry 3854*; 1160 m, *Lowry 4700.*

Growth rings absent in *A. glabratum* [3375] (Fig. 1) and *A. velutinum* [4700], and distinctly marked by diffuse-in-aggregates and marginal axial parenchyma forming tangential lines and narrow bands near their boundaries (Fig. 2) in other specimens.

Vessels rounded to slightly angular in outline, narrow to moderately wide (tangential diameter (36–)66–87(–152) μm), mostly in radial multiples of 2 to 4, not numerous (11 to 26 per mm² in *A. velutinum* [4700]; and 27 to 50 per mm² in other samples). Vessel walls 2–7 μm thick. Tyloses not observed. Vessel element length (320–)620–820(–1140) μm. Perforation plates simple (more than 50%), and scalariform with few bars (up to 18 in *A. glabratum* [4798]), and reticulate (Figs. 9 and 10), rarely double, in ± oblique end walls. Intervessel pits alternate (Fig. 11), rarely opposite to scalariform, 3–5(–6) μm in vertical diameter, rounded or oval with lens- to slit-like apertures. Vessel-ray and vessel-axial parenchyma pits with distinct borders; similar to intervessel pits in size and shape (mostly scalariform in *A. glabratum* [4798]), or unilaterally compound (horizontally to vertically elongated pits on the ray cell walls abut 2 to 5 pits on the vessel walls), with lens- to slit-like apertures surrounded by shallow, groove-like wall sculptures (Fig. 12). Helical thickenings absent.

Vasicentric and vascular tracheids not observed.

Fibers libriform, thick- to very thick-walled (5–22 μm), non-septate, with few simple to minutely bordered pits with slit-like apertures in radial walls.

Axial parenchyma scanty in *A. glabratum* [3375] and *A. velutinum* [4700], somewhat more abundant in other specimens, both paratracheal (appears mostly as solitary parenchyma cells in *A. glabratum* [3375] and *A. velutinum* [4700], or incomplete parenchyma sheaths near vessels in others) and apotracheal (diffuse in *A. glabratum* [3375] (Fig. 1) and *A. velutinum* [4700], diffuse-in-aggregates or marginal parenchyma (Fig. 2) in both other specimens). Strands composed of (2)3 to 5(7) cells.

Rays (3)4 to 6(10) per mm, uni- and multiseriate, mostly 3 or 4 cells wide in *A. velutinum* (Fig. 4), and 4 or 5 cells wide in *A. glabratum* (Fig. 3) (up to 8 cells wide in *A. glabratum* [4798]). Ray height commonly exceeding 1 mm in *A. glabratum* (up to 2.9 mm high in *A. glabratum* [4798]), and usually less than 1 mm in *A. velutinum.* Multiseriate rays formed mostly by square and procumbent cells (the latter more numerous in *A. velutinum*), with 1 to 3 (up to 6) marginal rows of upright cells, and usually with sheath cells of square to upright shape. Uniseriate rays composed of upright cells, rarely with some solitary square and procumbent cells. Pits on tangential walls of ray cells rounded and oval, very small (1–2 μm diam.). Radial canals absent.

Crystals (appearing mostly as a combination of few (1 to 3) large prismatic crystals with numerous small ones) common in ray cells (predominantly in square and upright ones) in *A. glabratum* [4798] and *A. velutinum* [4700], and in young parts of stem (near the pith) in *A. velutinum* [3854] (Fig. 8), occurring rarely in *A. glabratum* [3375]. Crystals present also in non-chambered axial parenchyma cells of *A. velutinum* [3854]. Brown and yellow deposits contained in a few vessels in both species examined.

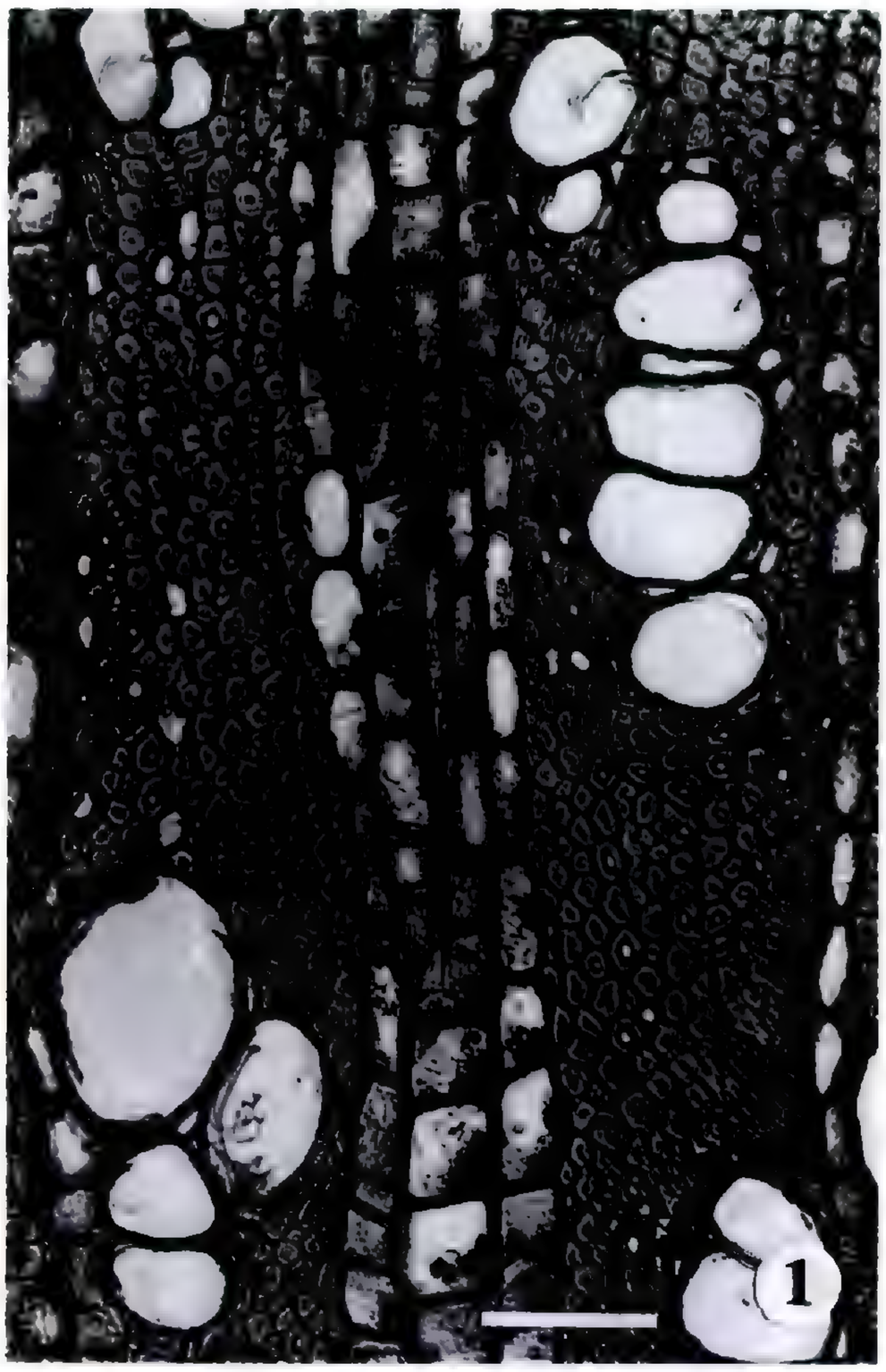
2. *MACKINLAYA* (FIGS. 5–7, 13–16; TABLES 1, 2).

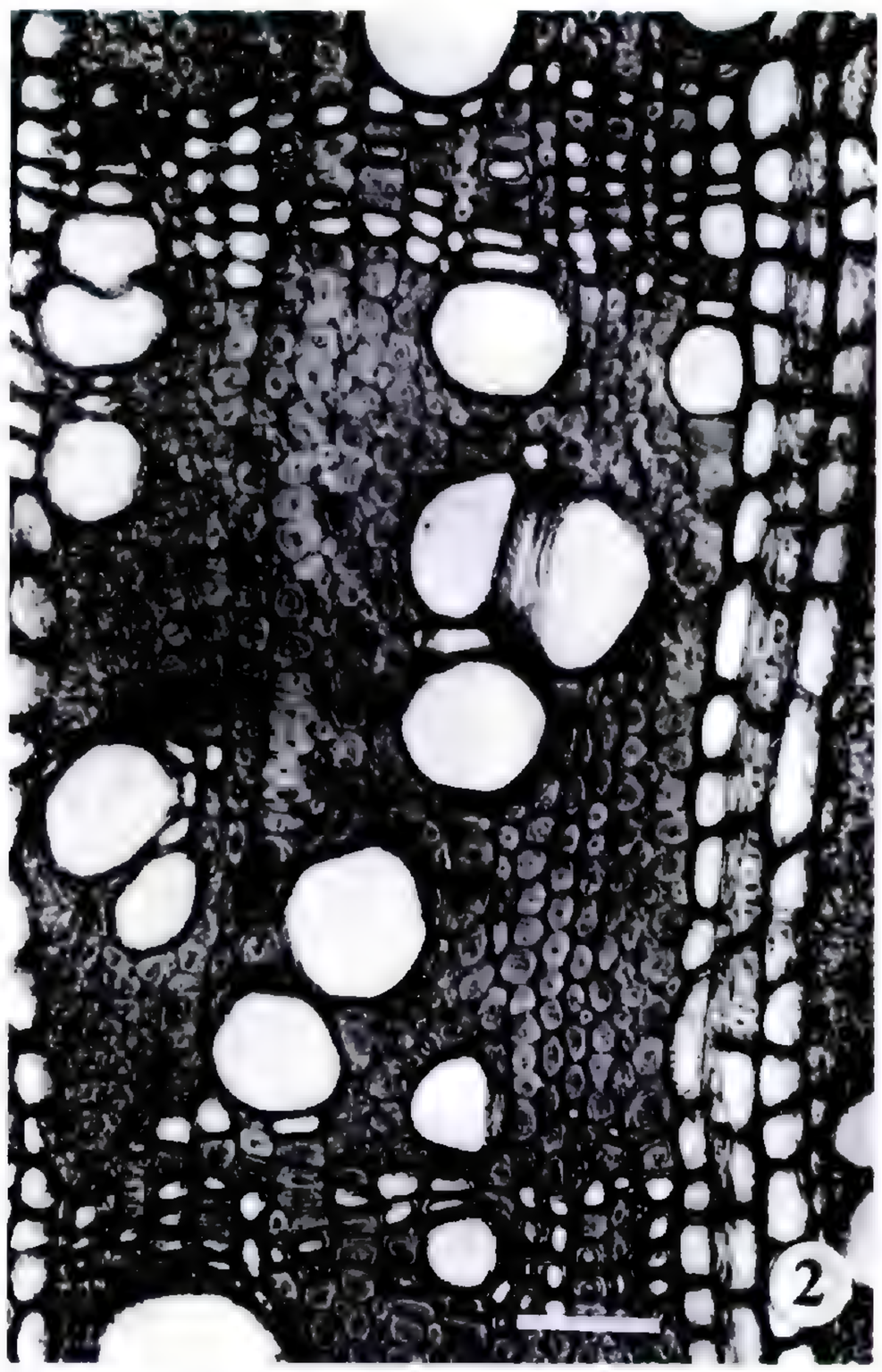
Material studied. Mackinlaya confusa Hemsl.: AUSTRALIA. Queensland: Bellenden Ker, 700 m, *Plunkett 1512*; Longlands Gap, 1120 m, *Plunkett 1520*; Isabella Falls, ca. 30 km NW of Cooktown, 180 m, *Plunkett 1549. M. macrosciadea* (F. Muell.) F. Muell.: AUSTRALIA. Queensland: without precise locality, 1100 m, *Hyland 15281*; Tolga, 800 m, *Plunkett 1497*; Gillies Lookout road, *Plunkett 1526*; cult. in Sydney Botanical Garden (NSW 208585), voucher for original collection: *Weston et al. 938* (AUSTRALIA. Queensland: Bellenden Ker, Mt. Bartle Frere; deposited at NSW).

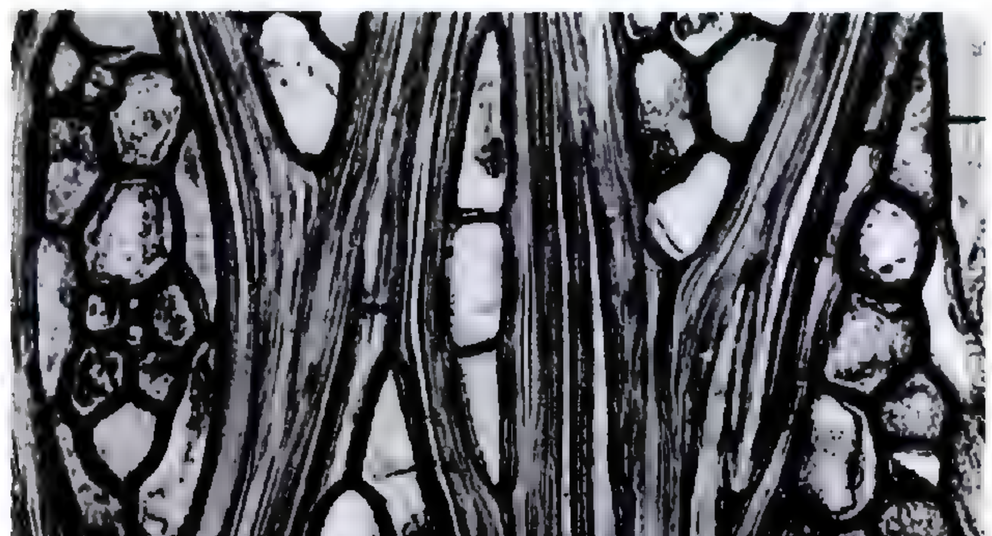
Growth rings absent or ± distinct (Fig. 5), marked by lines of marginal parenchyma.

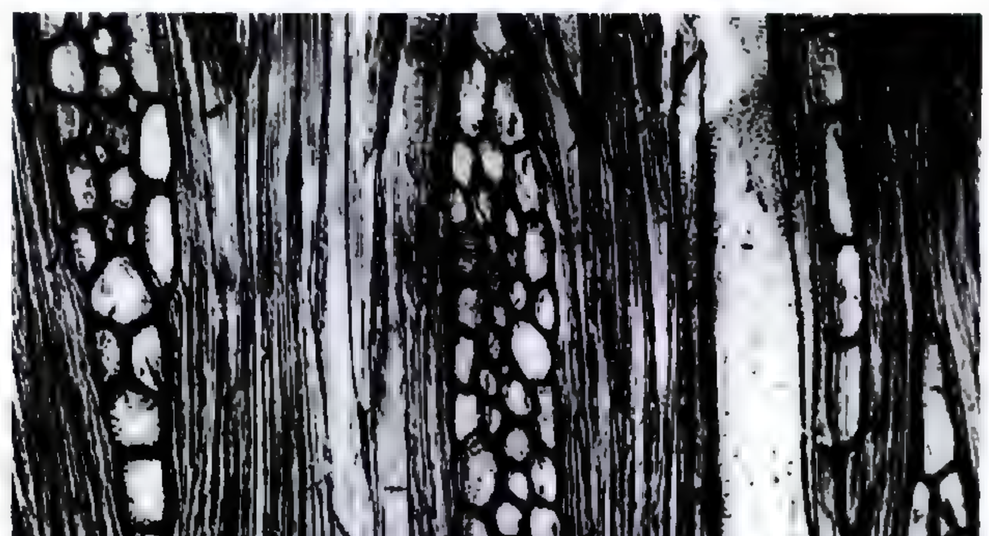
Vessels rounded, very narrow (tangential diameter 9–31 μm in *M. macrosciadea* [15281] and 20–52 μm in other samples), not numerous (20 to 44 per mm² in *M. confusa* [1512 and 1520]), to rather numerous (40 to 72 per mm² in *M. confusa* [1549] and *M. macrosciadea* [1526 and 938], to 70 to 107 per mm² in *M. macrosciadea* [1497 and 15281]), solitary and in radial multiples of 2 to 5 (up to 17 in *M. macrosciadea* [15281]). Vessel walls 2–5(–8) μm thick. Tyloses not observed. Vessel element length (270–)520–770(–1024) μm. Perforation plates scalariform with few (up to 14) bars and also rarely simple in *M. confusa* (observed in *M. confusa* [1549] only), or mostly simple (Fig. 14) and sometimes scalariform with few (up to 6) bars (Fig. 13) in *M. macrosciadea,* occasionally reticulate, in oblique and horizontal end walls. Intervessel pits transitional to alternate (transitional ones more common in *M. macrosciadea* [1497 and 1526], and *M. confusa* [1512]), rarely opposite, 3–6 μm in vertical diameter, rounded or oval with slit- to lens-like apertures commonly surrounded by shallow, groove-like wall sculptures (Fig. 14). Vessel-ray and vessel-axial parenchyma pits with distinct borders, similar to intervessel pits in size and shape, or unilaterally compound (then horizontally to vertically elongated pits on the ray cell walls corresponding to 2 or 3 pits on the vessel walls). Helical thickenings absent.

Vasicentric and vascular tracheids not observed.

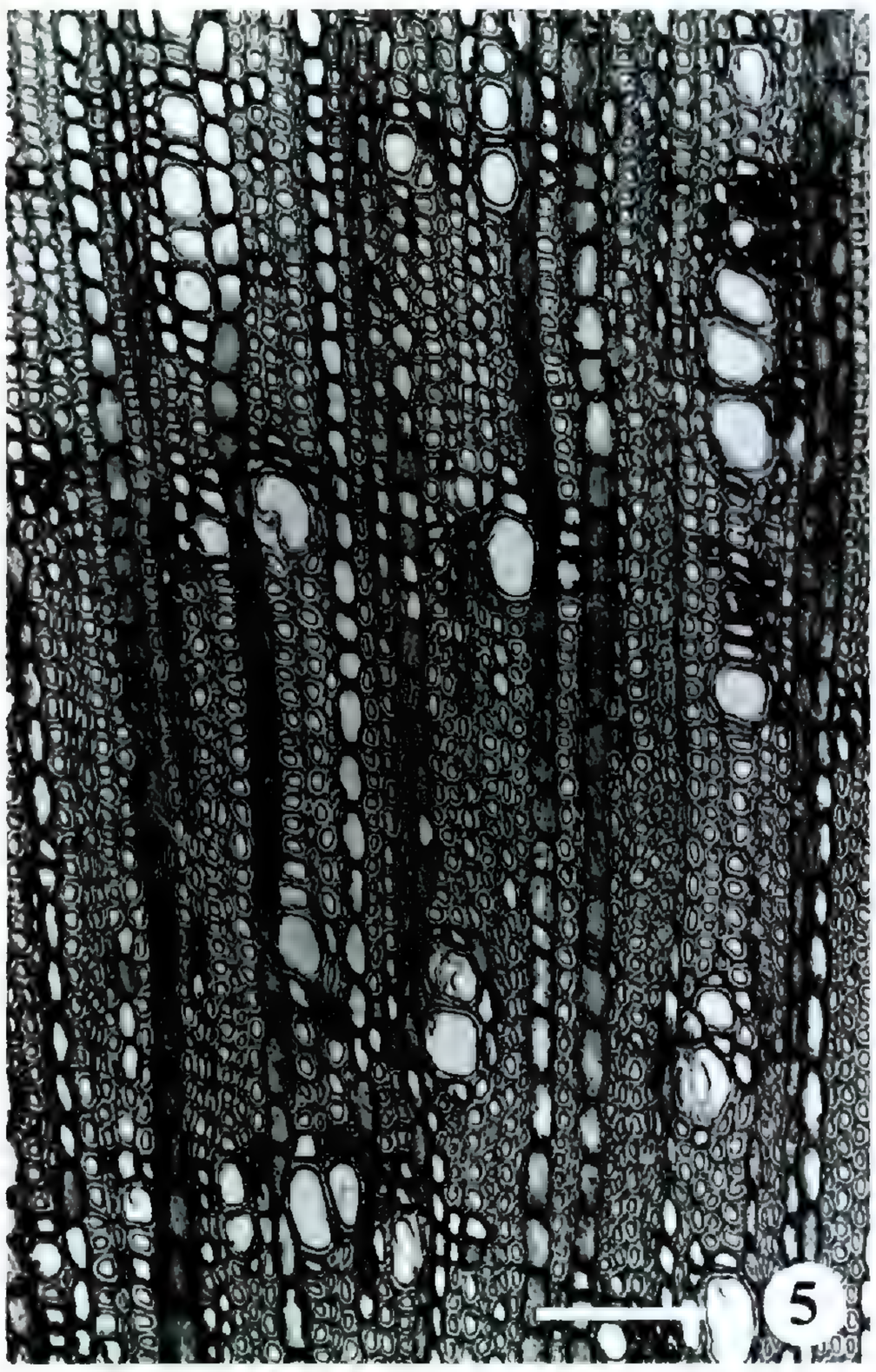








Figures 1–4. Light micrographs of *Apiopetalum* wood. —1. *A. glabratum*, *Lowry 3375*, transverse section, axial parenchyma scanty paratracheal and diffuse. —2. *A. velutinum*, *Lowry 3854*, transverse section, growth rings distinct, axial parenchyma scanty paratracheal and rather abundant diffuse-in-aggregates tending to form long tangential lines on growth ring boundaries. —3. *A. glabratum*, *Lowry 3375*, tangential section, mostly 4–5-seriate rays with sheath cells. —4. *A. velutinum*, *Lowry 3854*, tangential section, mostly 3–4-seriate rays with sheath cells. Scale bar = 100 μm.



Figures 5–8. Light micrographs of *Mackinlaya* and *Apiopetalum* wood. —5. *M. macrosciadea, Hyland 15281,* transverse section, growth rings absent, axial parenchyma scanty paratracheal and diffuse. —6. *M. macrosciadea, Hyland 15281,* tangential section, 1–3-seriate rays composed mostly of square and upright cells, multiseriate portions of some rays as wide as uniseriate ones and alternate with the latter. —7. *M. macrosciadea, Weston et al. 938,* radial section, helical thickenings on the inner walls of the ray cells (arrows). —8. *A. velutinum, Lowry 3854,* radial section, crystals (arrow) in a ray cell in the young part of the stem (near the pith). Scale bar = 100 μm.

Table 1. Data on wood samples examined and anatomical characters of vessel elements in *Apiopetalum* and *Mackinlaya*.

	Radius of sample 1	Radius of pith 2	Length of vessel elements 3	Diameter of vessels 4	Percentage of solitary vessels 5	Maximum vessel number per group 6	Vessel frequency 7	Number of bars per perforation plate 8
Apiopetalum glabratum Baill., *Lowry 3375*	34	4	663 ± 15.5 (710–1350)	87 ± 2.6 (48–152)	21	8	35 (31–50)	0 (0–15)
A. glabratum Baill., *Lowry 4798*	41	3	651 ± 38.1 (320–1140)	74 ± 1.1 (56–92)	17	7	31 (27–32)	0 (0–18)
Apiopetalum velutinum Baill., *Lowry 3854*	15	4	782 ± 19.4 (570–1000)	66 ± 1.6 (36–104)	46	4	32 (30–49)	0 (0–16)
A. velutinum Baill., *Lowry 4700*	34	3	822 ± 41.0 (460–1140)	80 ± 2.0 (52–128)	57	4	19 (11–26)	0 (0–14)
Mackinlaya confusa Hemsl., *Plunkett 1512*	11	1	770 ± 29.0 (520–1020)	36 ± 0.8 (20–48)	22	6	33 (27–44)	7 (3–14)
M. confusa Hemsl., *Plunkett 1520*	9	1	607 ± 32.0 (370–920)	30 ± 0.8 (20–44)	23	5	25 (20–35)	7 (3–9)
M. confusa Hemsl., *Plunkett 1549*	9	1	718 ± 24.8 (530–960)	36 ± 0.7 (24–52)	10	11	50 (42–69)	4 (0–12)
Mackinlaya macrosciadea (F. Muell) F. Muell *Plunkett 1497*	9	2	635 ± 17.0 (460–820)	32 ± 0.9 (20–48)	9	11	82 (70–107)	1 (0–4)
M. macrosciadea (F. Muell) F. Muell *Plunkett 1526*	7	2	663 ± 16.2 (550–800)	33 ± 0.8 (20–48)	10	12	60 (47–72)	1 (0–6)
M. macrosciadea (F. Muell) F. Muell, *Hyland 15281*	5	1	572 ± 28.3 (270–810)	21 ± 0.6 (9–31)	8	17	95 (79–102)	0 (0–5)
M. macrosciadea (F. Muell) F. Muell, *Weston et al. 938*	6	1	518 ± 28.8 (330–770)	30 ± 0.8 (20–44)	25	5	52 (40–69)	0 (0–5)

Characters: 1, Radius of wood sample (mm); 2, Radius of pith (mm); 3, Length of vessel elements (μm): average ± standard error (minimum − maximum); 4, Tangential diameter of vessels (μm): average ± standard error (minimum − maximum); 5, Percentage of solitary vessel lumina; 6, The greatest number of vessels in a vessel group; 7, Vessel frequency (per mm^2): average (minimum − maximum); 8, Number of bars per perforation plate: average (minimum − maximum).

Table 2. Anatomical characters of libriform fibers and ray parenchyma in *Apiopetalum* and *Mackinlaya*.

	Length of fibers 1	Width of rays 2	Height of rays 3	Number of uniseriate rays 4	Number of multiseriate rays 5
Apiopetalum glabratum Baill., *Lowry 3375*	1029 ± 25.4 (710–1350)	4.3/6	1.1/2.9	0.8 (0–2)	3.0 (2–5)
A. glabratum Baill., *Lowry 4798*	1267 ± 26.4 (970–1840)	3.8/8	1.1/1.9	0.7 (0–2)	4.0 (2–5)
Apiopetalum velutinum Baill., *Lowry 3854*	1196 ± 26.8 (920–1510)	3.8/6	0.8/1.2	1.2 (0–2)	3.6 (2–5)
A. velutinum Baill., *Lowry 4700*	1074 ± 23.1 (810–1390)	3.3/5	0.6/1.2	0.5 (0–1)	3.6 (2–5)
Mackinlaya confusa Hemsl., *Plunkett 1512*	1324 ± 28.3 (870–1610)	1.3/3	0.8/3.1	10.5 (7–15)	2.4 (1–3)
M. confusa Hemsl., *Plunkett 1520*	1293 ± 27.6 (870–1620)	1.3/3	1.4/3.6	14.0 (12–16)	2.5 (1–5)
M. confusa Hemsl., *Plunkett 1549*	1076 ± 32.2 (730–1530)	2.1/5	1.7/3.6	8.6 (6–12)	4.5 (4–6)
Mackinlaya macrosciadea (F. Muell) F. Muell *Plunkett 1497*	1022 ± 21.0 (730–1300)	2.2/6	1.1/2.6	7.2 (3–12)	4.7 (3–6)
M. macrosciadea (F. Muell) F. Muell *Plunkett 1526*	1045 ± 22.2 (780–1310)	1.6/4	0.9/2.1	10.2 (4–14)	4.2 (2–7)
M. macrosciadea (F. Muell) F. Muell, *Hyland 15281*	801 ± 15.3 (620–1040)	1.5/3	0.7/1.1	13.9 (6–21)	4.7 (2–8)
M. macrosciadea (F. Muell) F. Muell, *Weston et al. 938*	722 ± 24.0 (420–1060)	1.8/4	0.9/1.9	8.7 (4–11)	12.5 (10–14)

Characters: 1, Length of libriform fibers (μm): average ± standard error (minimum − maximum); 2, Width of rays: average/maximum number of cell; 3, Height of rays (mm): average/maximum; 4, Number of uniseriate rays per mm: average (minimum − maximum); 5, Number of multiseriate rays per mm: average (minimum − maximum).

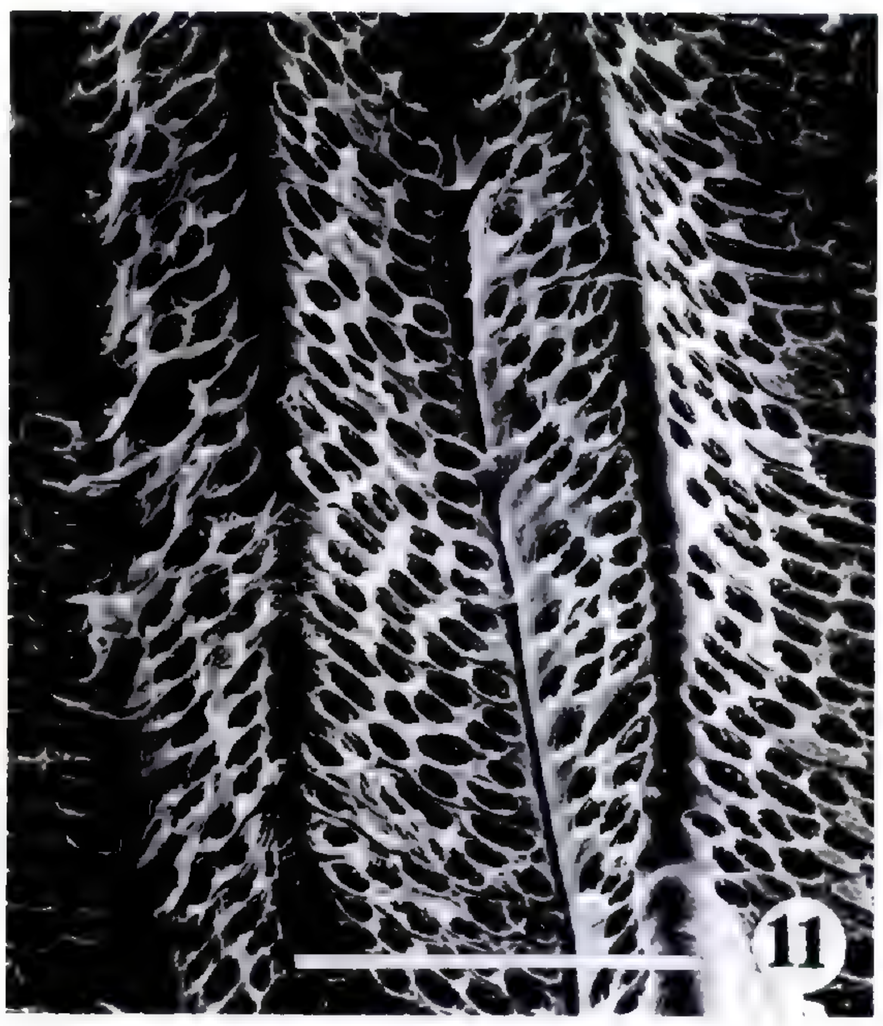
Fibers libriform, thin- to thick-walled (walls 3–5(–8) μm thick), non-septate, with rather numerous simple to minutely bordered pits with slit-like apertures in radial walls.

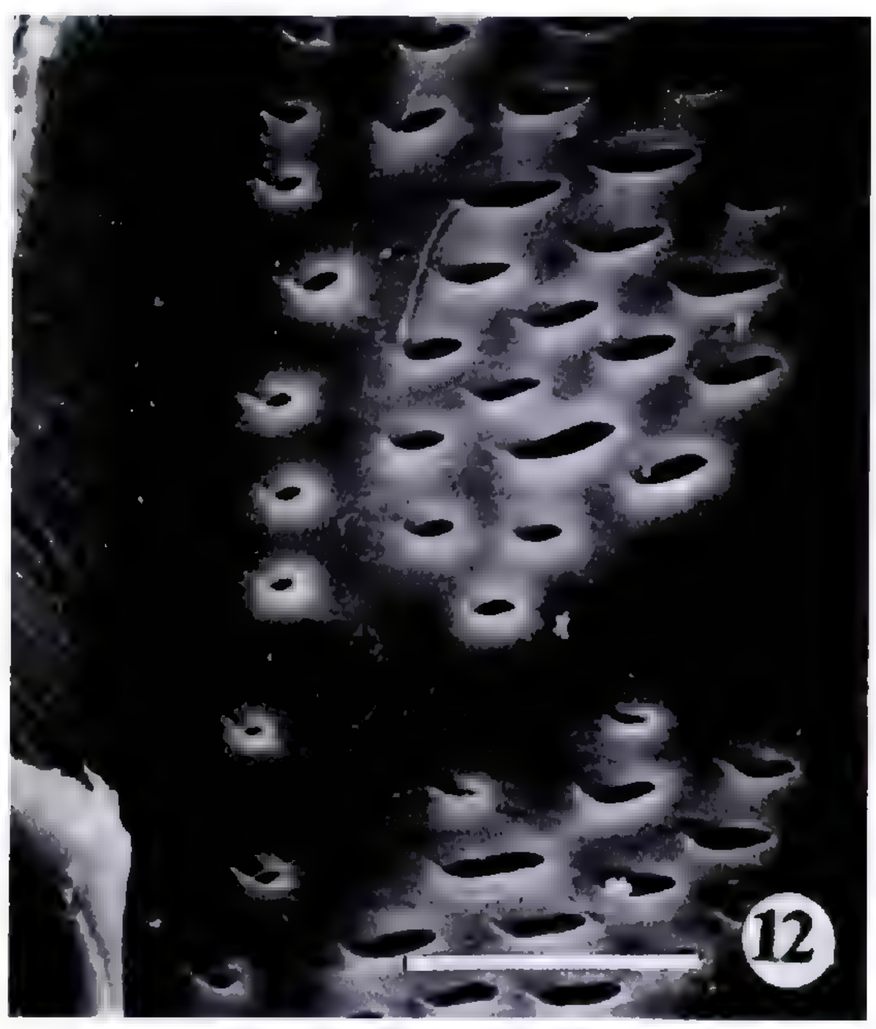
Axial parenchyma scanty paratracheal (appearing as solitary parenchyma cells near vessels), diffuse, and marginal, sometimes forming interrupted tangential lines (Fig. 5) and narrow bands near boundaries of the growth rings. Strands composed of 3 to 6(7) cells. Helical thickenings rarely present (*M. macrosciadea* [938]) on inner walls of the axial parenchyma cells in contact with vessels (Fig. 15).

Rays numerous ((14)18 to 21(24) per mm), uniseriate and multiseriate of 2 or 3 cells in width in *M. confusa* [1512 and 1520] and wider in other samples (up to 6 cells in *M. macrosciadea* [1497]). Uniseriate rays more numerous than multiseriate ones in all samples except *M. macrosciadea* [938]. Ray height commonly exceeding 1 mm in *M. confusa* [1512 and 1549], and in *M. macrosciadea* [1497], and commonly less than 1 mm in other samples. Uniseriate rays composed of upright and few square cells. Alternation of uniseriate and multiseriate portions common in same ray. Uniseriate portions of multiseriate rays very long (up to 20 rows), formed by upright and solitary square cells, multiseriate portions usually as wide as uniseriate ones, composed of square and a few procumbent cells (Fig. 6). Pits on tangential walls of ray cells rounded and oval, very small (1–2 μm diam.). Helical thickenings rarely present in *M. macrosciadea* [938] on inner walls of both upright/square and procumbent ray cells in contact with vessels (Figs. 7, 16). Radial canals absent. Crystals not observed. Brown and yellow deposits contained in a few to many vessels in both species examined, and also in cavities of many fibers and parenchyma cells of *M. macrosciadea* [938].

Discussion

Very little variation was observed in wood structure within the *Apiopetalum* and *Mackinlaya* species examined. *Apiopetalum glabratum* differs from *A. velutinum* by higher and wider rays (Table 2). *Mackinlaya confusa* is distinct from *M. macroscia-*





Figures 9–12. Scanning electron micrographs of *Apiopetalum* wood. —9. *A. velutinum, Lowry 3854,* reticulate perforation plate. —10. *A. velutinum, Lowry 3854,* radial section, note one scalariform and two simple perforation plates. —11. *A. glabratum, Lowry 3375,* alternate intervessel pitting. —12. *A. velutinum, Lowry 3854,* vessel-ray pitting with lens-like apertures surrounded by shallow, groove-like wall sculptures. Scale bar in Figures 9–11 = 50 μm, in Figure 12 = 20 μm.

dea by the predominance of scalariform perforation plates with more numerous bars; it also has less numerous vessel lumina, but more samples must be studied before the importance of this character can be interpreted.

Helical thickenings on the walls of both ray and axial parenchyma were observed rarely in one sample of *Mackinlaya macrosciadea* [938] (Figs. 7, 15, 16). This feature generally appears in tracheal elements (vessel elements, vascular/vasicentric tracheids, fibers), and has also been reported very rarely in axial parenchyma of some Trigoniaceae (Heimsch, 1942), Ancistocladaceae (Gottwald & Parameswaran, 1968), and Chrysobalanaceae (ter Welle, 1975). The presence of helical thickenings in ray cells appears to have been reported previously only for *Trigonia sericea* HBK (Heimsch, 1942: 133). In the sample of *Mackinlaya,* helical thickenings are found in parenchyma cells adjacent to the vessels.

Mackinlaya and *Apiopetalum* can be clearly distinguished from one another on the basis of their wood anatomy. Differences occur in several features, including narrower and more numerous vessels, thinner fiber walls, narrower and more numerous rays, the absence of diffuse-in-aggregates axial parenchyma, and the absence of crystals in the ray and axial parenchyma cells in *Mackinlaya* (Tables 1–3). The very narrow and relatively numerous vessels, and the 1- or 2-seriate rays found in *Mackinlaya* are probably correlated with the shrubby habit of the species studied and the correspondingly small di-

Figures 13–16. Scanning electron micrographs of *M. macrosciadea, Weston et al. 938,* wood. —13. Vessel element, scalariform perforation plates with few bars. —14. Simple perforation plate; small intervessel pits with slit- to lens-like apertures surrounded by shallow, groove-like wall sculptures. —15. Helical thickenings in an axial parenchyma cell. —16. Helical thickenings in a ray cell. Scale bar in Figure 13 = 50 μm; in Figures 14–16 = 20 μm.

ameter of their stems. Other shrubby Araliaceae such as species of *Oplopanax* and *Astrotricha* (Oskolski, 1994, 1996) have mean values of vessel diameter and frequency, as well as of ray width, similar to those observed in *Mackinlaya.* Thickness of fiber walls, the type of axial parenchyma, and the occurrence of crystals in parenchyma cells appear to reflect relationships more than differences in habit or habitat (Oskolski, 1994, 1996), and could be of possible taxonomic value (Table 3).

Despite these differences in their wood anatomy, *Mackinlaya* and *Apiopetalum* also share a number of significant features. These include the small intervessel pits (3–6 μm), the occurrence of both paratracheal and apotracheal (diffuse and diffuse-in-aggregates, the latter occurring only in *Apiopetalum*) axial parenchyma, non-septate fibers, a predominance of upright and square cells in the ray composition, and brown deposits in the vessels. Although each of these characters has also been observed in other genera of Araliaceae and Apiaceae, their combined occurrence in *Mackinlaya* and *Apiopetalum* is notable for the order Apiales (Table 3) and supports the suggestion that these two genera are closely related. Diffuse and diffuse-in-aggregates parenchyma is found alone only in species of *Myodocarpus, Delarbrea,* and *Pseudosciadium.* Co-occurrence of both diffuse and paratracheal axial parenchyma is known only from three New Caledonian species of the pan-tropical genus *Schefflera* (Oskolski & Lowry, in prep.). All other representatives of Apiales examined to date have only a single parenchyma type. The co-occurrence of apotracheal and paratracheal axial parenchyma could

Table 3. Wood anatomical diversity in *Apiopetalum*, *Mackinlaya*, other Araliaceae, and selected woody Apiaceae. Noteworthy character associations are outlined in boxes. For character states co-occurring in individual samples, all are listed; "+" = character present; "−" = character absent; "+/−" = character intermediate between two contrasting states.

	Vessels narrow	Size of intervessel pits	Fiber wall thickness	Septate fibers	Axial parenchyma type	Ray type	Radial canals	Crystals
	1	2	3	4	5	6	7	8
Apiopetalum glabratum	-	x	xxx	-	P, M, D, Da	IIA	-	+
A. velutinum	-	x	xxx	-	P, M, D, Da	IIA	-	+
Mackinlaya confusa	+	x	x	-	P, M, D	IIA	-	-
M. macrosciadea	+	x	x	-	P, M, D	IIA	-	-
Other Araliaceae[1]								
Myodocarpus	+/-	x	xxx	+/-	D, Da	Ho	+	-
Delarbrea	+/-	x	xxx	-	D, Da	Ho	+	-
Pseudosciadium	+	x	xxx	-	D, Da	Ho	+	-
Astrotricha	+	x	x	-	P	Pm	-	-
Dendropanax[2]	+/-	xx	x	+	P	IIB	+	-
Woody Apiaceae[1]								
Bupleurum	+	xx	xx	?	P	IIB	-	-
Heteromorpha	+/-	?	x	?	P	IIB	-	?
Steganotaenia	-	?	x	?	P, M	IIB	+	?
Myrrhidendron	+/-	?	x	?	P	IIB	-	-
Eryngium	+/-	?	x	?	P	IIB	+	?
Gymnophyton	+/-	?	x	?	P, M	IIB	-	?
Asteriscum	+/-	?	x	?	P, M	?	-	?
Trachymene	+	?	x	?	P	IIB	-	?

Characters: 1, Vessel lumina narrow (diameter < 50 μm); 2, Size of pits of the intervessel pitting (x = up to 8 μm, xx = more than 8 μm in vertical diameter); 3, Libriform fiber wall thickness (x = thin, xx = thin to thick, xxx = very thick); 4, Presence of septate fibers; 5, Axial parenchyma types (P = scanty paratracheal, M = marginal, D = diffuse, Da = diffuse-in-aggregates); 6, Ray type (I, IIA, IIB = Kribs's (1935) types of heterogeneous rays, Ho = homogeneous I rays, Pm = "paedomorphic" rays (Carlquist, 1988), which are composed of square and upright cells only); 7, Presence of radial canals; 8, Presence of crystals in ray and/or axial parenchyma cells.

Notes: 1. Data on genera of Araliaceae other than *Apiopetalum* and *Mackinlaya* are from Oskolski et al. (1997) for *Myodocarpus*, *Delarbrea*, and *Pseudosciadium*; and Oskolski (1996) for *Astrotricha* and *Dendropanax*. Data on woody Apiaceae are from Rodriguez (1957), Metcalfe & Chalk (1950), Greguss (1959), and Schweingruber (1990). 2. Data on *Dendropanax* are presented as an example of the wood characters typical for most Araliaceae.

thus be regarded as an apomorphy for *Mackinlaya* and *Apiopetalum*.

Besides diffuse and/or diffuse-in-aggregates axial parenchyma, several other wood-anatomical features are also shared among *Mackinlaya*, *Apiopetalum*, and the assemblage comprising *Myodocarpus*, *Delarbrea*, and *Pseudosciadium*, such as the presence of small intervessel pits. This character occurs nowhere else within Araliaceae except in the Australian endemic *Astrotricha* (Table 3), which may also be a basally branching lineage of Apiales as indicated by recent molecular sequence data from ITS (Mitchell & Wagstaff, 1997; G. M. Plunkett, pers. comm.). However, the wood of *Astrotricha* differs strongly from that of *Mackinlaya* and *Apiopetalum*, notably in its axial parenchyma and ray types, and the presence of helical thickenings on the vessel walls (Oskolski, 1996). Species of *Mackinlaya*, *Apiopetalum*, *Myodocarpus*, *Delarbrea*, and *Pseudosciadium* also form very thick-walled non-septate fibers, which are unusual within the order. Each of these wood characters may be a synapomorphy within Apiales (Table 3), supporting the hypothesis of monophyly of the alliance that contains these five genera. They could likewise be symplesiomorphic in that they occur independently (but not together) in other groups within the order (Table 3), suggesting the possibility of parallel evolution. Resolving this issue is difficult at present because we lack sufficient data on wood features (especially the size of intervessel pits) for nearly all genera of woody Apiaceae.

These similarities notwithstanding, wood anatomy of *Mackinlaya* and *Apiopetalum* is nevertheless very distinct from that of *Myodocarpus*, *Delarbrea*, and *Pseudosciadium*. In addition to the co-occurrence of both paratracheal and apotracheal (diffuse and diffuse-in-aggregates) axial parenchyma, ray types also differ. In *Mackinlaya* and *Apiopetalum*, rays are heterogeneous, with both upright and square as well as procumbent cells; they are homogeneous with exclusively procumbent cells in the other genera. Furthermore, *Mackinlaya* and *Apiopetalum* have brown and yellow deposits in their vessels and lack radial canals. Wood anatomy thus supports the inclusion of these genera in a monophyletic Mackinlayeae (Table 3), and refutes the inclusion of *Pseudosciadium* (Baillon, 1878, 1879), which is most closely related to *Myodocarpus* and especially *Delarbrea* (Lowry, 1986a, b; Oskolski et al., 1997; Plunkett, 1998, unpublished data).

Using generally accepted trends in wood evolution (Bailey & Tupper, 1918; Frost, 1930a, b, 1931; Carlquist, 1988; Baas & Wheeler, 1996) to determine character polarity, several features are regarded as plesiomorphic for *Mackinlaya* and *Apiopetalum*. These include predominantly scalariform perforation plates and relatively long vessel elements, whereas short vessel elements with exclusively simple perforation plates of woody Apiaceae (Metcalfe & Chalk, 1950; Rodriguez, 1957; Greguss, 1959; Schweingruber, 1990) are regarded as apomorphic. Average vessel element lengths are 651–822 μm in *Apiopetalum* and 518–770 μm in *Mackinlaya* (Table 1), within the range seen in most other Araliaceae, which vary from 650 to 900 μm. The lowest reported averages are 366 μm in *Oplopanax horridum* (J. Smith.) Miq. and 374 μm in *Eleutherococcus sessiliflorus* (Rupr. & Maxim.) S. Y. Hu, with the highest of 1339 μm reported in *Schefflera gabriellae* Baill. (Oskolski, 1994, 1996; Oskolski & Lowry, in prep.). By contrast, vessel elements in *Apiopetalum* and *Mackinlaya* are distinctly longer than in woody Apiaceae, which have average values that are generally less than 400 μm. A notable exception is *Heteromorpha arborescens* (Thunb.) Cham. & Schlecht., whose average vessel length reaches 502 μm (Rodriguez, 1957). Wood features characteristic of *Mackinlaya* and *Apiopetalum*, such as the occurrence of diffuse and diffuse-in-aggregates apotracheal parenchyma types, small intervessel pits, and heterogeneous rays with distinct uniseriate portions composed of upright and square cells (Kribs's (1935) IIA type), have not been reported among the woody Apiaceae examined, including species of *Bupleurum*, *Heteromorpha*, *Steganotaenia*, *Myrrhidendron*, *Eryngium*, *Gymnophyton*, *Asteriscum*, and *Trachymene* (Table 3), among others (Metcalfe & Chalk, 1950; Rodriguez, 1957; Greguss, 1959; Schweingruber, 1990).

Wood anatomical features thus confirm that *Mackinlaya* and *Apiopetalum* are closely related and occupy an isolated position within Apiales, as proposed by Plunkett (1998). However, wood characters neither validate nor refute the hypothesis that the two genera under study are intermediate between Araliaceae and Apiaceae (Philipson, 1970; Rodriguez, 1957, 1971), nor do they offer a clear indication of the group's phylogenetic position within the order. Based on current data, we cannot identify any reliable synapomorphies in the wood to support a sister relationship between the *Apiopetalum–Mackinlaya* clade and core Apiaceae (excluding most members of subfamily Hydrocotyloideae), as suggested by Plunkett (1998), nor between them and core Araliaceae (excluding *Myodocarpus*, *Delarbrea*, and *Pseudosciadium*). However, the study of wood structure is of limited use in assessing relationships between *Mackinlaya* and *Apiopetalum* and non-woody members of the *Mack-*

inlaya group, as defined by Plunkett (1998, pers. comm.), or between them and nearly all of the other genera of Apiaceae, which are likewise herbaceous. Several wood characters (small intervessel pits, thick fiber walls, non-septate fibers) do suggest a relationship between *Mackinlaya* and *Apiopetalum* and the alliance comprising *Myodocarpus*, *Delarbrea*, and *Pseudosciadium*, as previously hypothesized (Plunkett et al., 1996a, b, 1997).

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CARLOS SPEGAZZINI (1858–1926): TRAVELS AND BOTANICAL WORK ON VASCULAR PLANTS[1]

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ABSTRACT

Carlos Luis Spegazzini (1858–1926) was a leading figure in Argentinian natural history, mainly recognized for his mycological and vascular plant studies. Information concerning Spegazzini's travels and his botanical work focusing on vascular plants, including his specimens and publications, is provided. The collecting expeditions of Spegazzini and collaborators in Argentina are summarized. The identification of his typic and non-typic materials is frequently complicated by the lack or sparcity of label information. Spegazzini published ca. 100 papers on vascular plants, mostly in Argentinian journals, describing ca. 1000 new taxa. A list of the new genera, species, and infraspecific taxa described by Spegazzini is provided. Spegazzini was professor at the University of La Plata and Buenos Aires in Argentina, curator of the herbarium of the National Department of Agriculture, first head of the herbarium of Museo de La Plata, and founder of an arboretum and an institute of mycology in La Plata city.

Key words: Argentina, botanist, plant collections, Spegazzini, travels, vascular plants.

Carlo Luigi Spegazzini, or "Carlos Luis" Spegazzini (the Spanish translation of his name by which he was recognized in Argentina and in the rest of the world), was a leading figure in Argentinian natural history, for both his mycological and vascular plant studies. Spegazzini was one of the most significant explorers of Patagonia in terms of the volume of his collections. He also traveled and collected extensively in almost all of Argentina, from northern Salta to southern Tierra del Fuego.

This botanist, who was attracted by the South American biota since he was a young student in Italy (Spegazzini, 1884a), is best known for his work in mycology, although his vascular plant systematics is of equivalent importance. His herbarium of vascular plants, collected by himself and by other collectors, reaches 100,000 specimens (Molfino, 1929), and he described approximately 1000 new taxa during his career. In the process, Spegazzini published more than 320 papers, of which ca. 100 refer to vascular plants. Furthermore, he was a teaching professor of botany, zoology, mineralogy, geology, and phytopathology, curator of the Ministerio de Agricultura de la Nación herbarium, first head of the herbarium of Museo de La Plata, and founder of the Arboretum of the Facultad de Ciencias Agronómicas y Forestales in La Plata city.

Biographies of Carlos Spegazzini considering his mycological work have been published previously (Scala, 1919; Hauman, 1923; Molfino, 1929, 1951; Arambarri & Spinedi, 1996), but details of his travels and botanical work on vascular plants are still undocumented. This information, including his specimens and publications, is provided herein.

BIOGRAPHICAL SKETCH

A detailed biography of Carlos Spegazzini has been published by José Molfino (1929), a botanist who married one of Spegazzini's daughters. We present here a brief outline of Spegazzini's life, as it pertains to his botanical endeavors.

Carlo Luigi Spegazzini (Fig. 1) was born on 20 April 1858 at Bairo in Torino, in northern Italy. He took courses in the School of Viticulture and Enology in Conegliano, Venice (Italy), where he graduated in 1879. There, he met Pier Andrea Saccardo (1845–1920), professor and founder of the *Scuola micologica di Padova* (Lazzari, 1973), who would be a major influence in Spegazzini's scientific career. Soon after graduating, thinking that botani-

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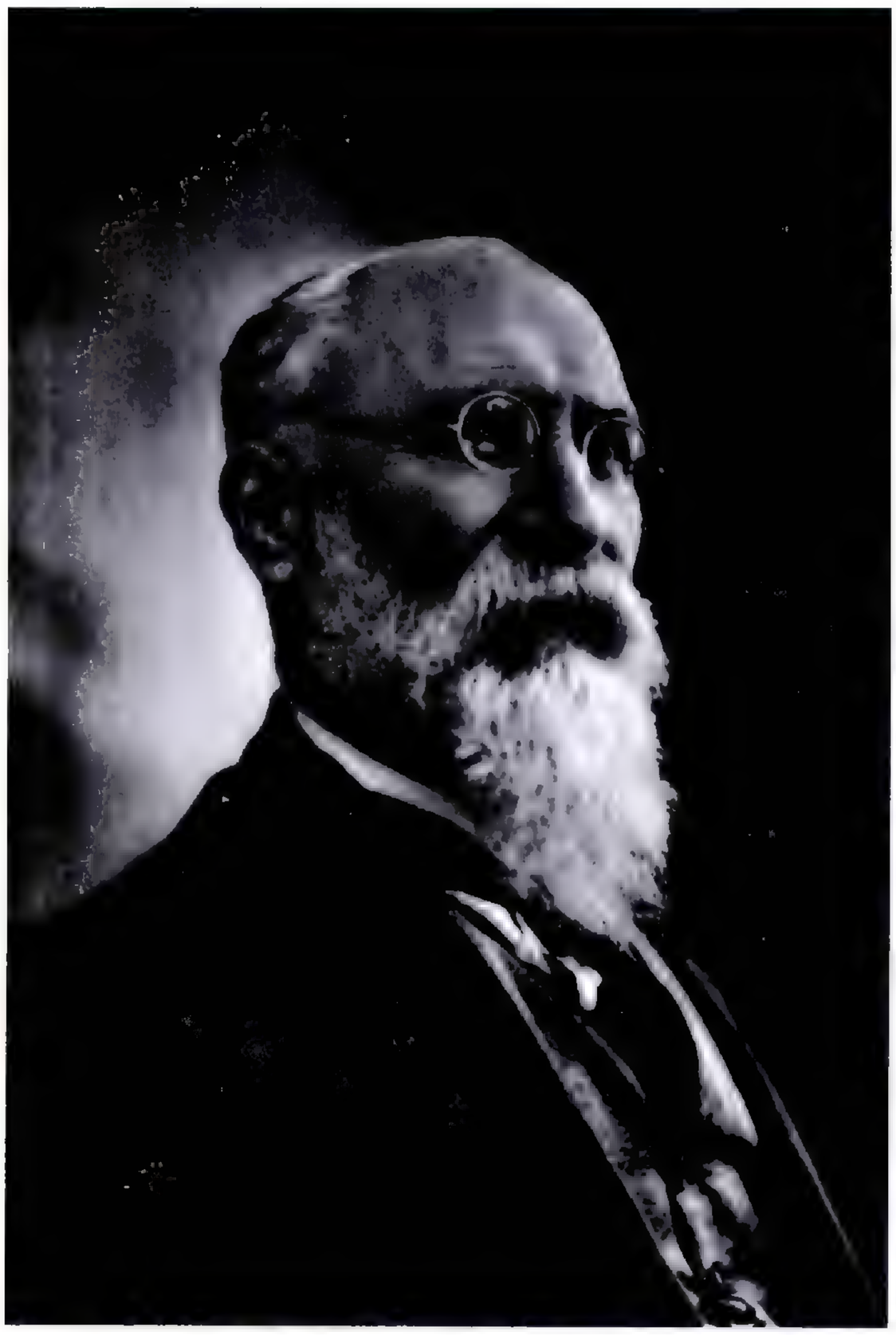

Figure 1. Carlo Luigi Spegazzini in a photograph taken on 20 April 1922, the day of his 65th birthday.

cal studies in Italy were well covered and completed by other Italian scientists, Spegazzini decided to move to another land. In December 1879, he disembarked in Buenos Aires harbor. It is interesting to note that the period from 1850 to 1923 was characterized by the arrival in Argentina of a large number of European naturalists (Ringuelet, 1960; Ragonese, 1986). They came mainly from Germany (Hermann Burmeister in 1857, Paul Lorentz and Georg Hieronymus in 1870, Fritz Kurtz in 1884), Italy (Domingo Parodi in 1875, Spegazzini in 1879, Augusto Scala in 1909), Russia (Carl Berg in 1873, Nikolai Alboff in 1895), Switzerland (Theodor Stuckert in 1869), and Belgium (Lucien Hauman in 1904).

The Spegazzini story in Argentina evolves from two fortunate circumstances: his unbounded enthusiasm for the natural sciences, and a scientifically young country waiting to be discovered. In the year after arriving he made his first field trip and published his first new species of vascular plant, *Cabomba australis* Speg. (Nymphaeaceae) (Spegazzini, 1880). In 1885, he was appointed professor in the Instituto Agronómico de Santa Catalina (Buenos Aires Province), and professor and temporary dean in the Facultad de Agronomía (University of La Plata). Two years later, he was designated Head of the Department of Botany in Museo de La Plata. At the same time, Spegazzini was a student at the School of Agriculture, obtaining his degree on 23 December 1897 (Ragonese, 1986).

In 1898 Spegazzini was designated curator of the Herbarium of the Ministerio de Agricultura de la Nación, in Buenos Aires (currently the Herbarium of the Instituto de Recursos Biológicos, INTA), and in the following year he founded the Arboretum of the Facultad de Agronomía in La Plata. In 1900 he was appointed Professor in the Facultad de Farmacia y Bioquímica, at the University of La Plata. He was a leader in the study of agriculture in the country. In his research in this field in Argentina, he discovered a fungus that attacks grape vines (*Plasmospora viticola*), the bacterium that causes a disease of sugar cane (*Bacillus sacchari*), and fungi that attack tobacco (*Peronospora nicotianae*) and alfalfa plants (*Uromyces striatus*).

During the years 1912 to 1914 he returned to Europe (Spegazzini, 1916a) to work in herbaria and also to visit his mentor and friend Pier Andrea Saccardo. One of his last botanical activities on 1 April 1925, was the foundation of a scientific journal in Argentina, *Revista Argentina de Botánica.* [Unfortunately, after Spegazzini's death the journal ceased publication.]

On 1 July 1926, at the age of 68, Carlos Spegazzini died in his home at La Plata from kidney disease. In his will he donated the house to science with the condition that it become an institute of botany bearing his name (currently *Instituto de Botánica Carlos Spegazzini,* LPS; Holmgren et al., 1990). He also donated his library (ca. 6000 books and papers), scientific instruments, and personal herbaria of fungi and vascular plants (Anonymous, 1930).

Collecting Expeditions

Carlos Spegazzini was an active field worker throughout his life. His principal expeditions and collecting efforts are outlined in Table 1 and Figures 2 and 3. Only the most representative of his frequent collecting trips around the cities of La Plata and Buenos Aires are cited in Table 1.

Spegazzini collected and also received specimens from other collectors (see Table 1) and his own children, Etile, Propile, and Rutile Spegazzini. His preferred area of collecting was Patagonia, the southern tip of Argentina including Mendoza, Neuquén, Río Negro, Chubut, Santa Cruz, and Tierra

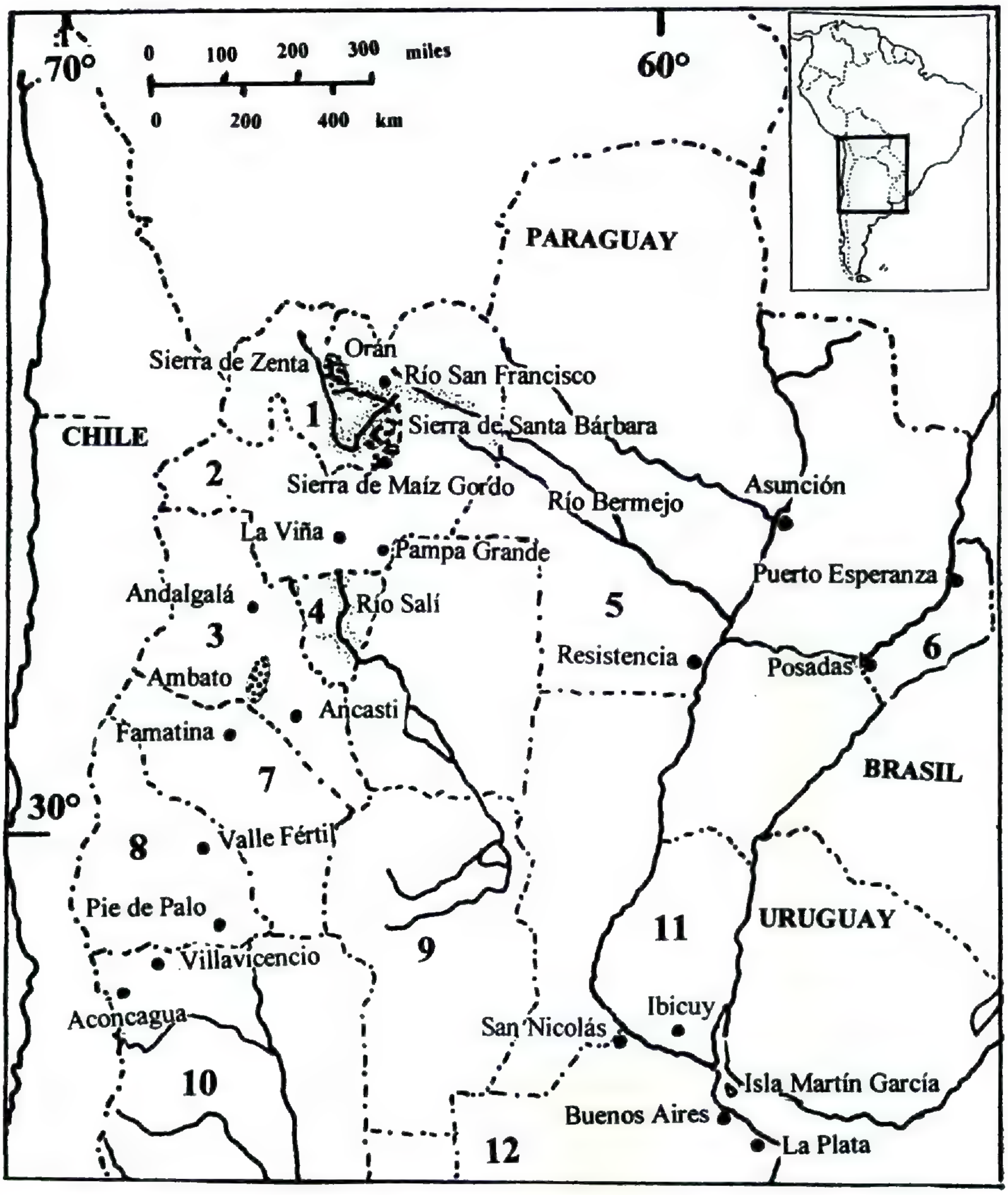

Figure 2. Map of northern and central Argentina with the main localities (mentioned in Table 1) of Spegazzini's and other collectors' vascular plant collections that contributed to his herbarium. Numbers refer to the Argentine provinces: 1 = Jujuy; 2 = Salta; 3 = Catamarca; 4 = Tucumán; 5 = Chaco; 6 = Misiones; 7 = La Rioja; 8 = San Juan; 9 = Córdoba; 10 = Mendoza; 11 = Entre Ríos; 12 = Buenos Aires.

del Fuego (Fig. 3). The countries adjacent to Argentina, on the other hand, were scarcely visited for collecting.

One of the first remarkable trips Spegazzini made was in 1881, two years after his arrival in Argentina. On December 18 he embarked as a botanist, representing the University of Buenos Aires, in the expedition of Lieutenant Santiago Bove aboard the ship *Cabo de Hornos*. Spegazzini, as well as a geologist, a geographer, and a zoologist, departed from Buenos Aires heading for Isla de los Estados and Tierra del Fuego, the southernmost part of America. After arriving at Punta Arenas, Chile, Spegazzini, Bove, and the geologist embarked in a smaller ship to sail through the narrow channels of Tierra del Fuego. A storm caused their shipwreck, but Spegazzini saved some of the plant collections by swimming to the coast and burying them in the snow to preserve them. While the three survivors awaited rescue, Spegazzini contacted the Indians of the area and learned the grammar of their language (Spegazzini, 1884b). Spegazzini returned to Buenos Aires on 27 September 1882. As a result of this adventure, Spegazzini collected 1108 specimens (mosses, fungi, lichens, and vascular plants). Forty-two years later, in January 1924, he returned to Cabo de Hornos in Chile where he discovered very different conditions. The Native Indian populations had almost disappeared, and the fauna and flora were reduced due to pressure from the increasing

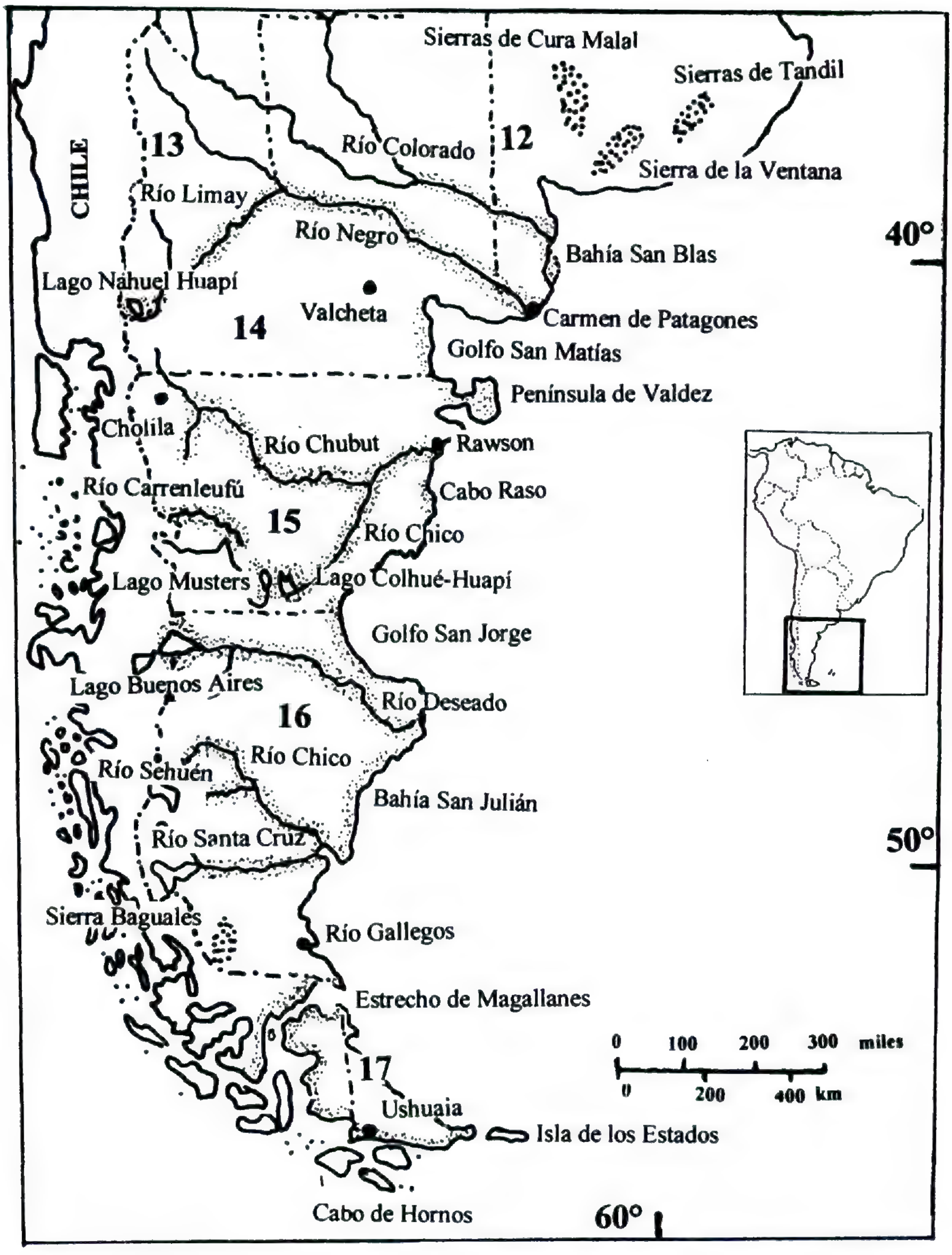

Figure 3. Map of southern Argentina with the main localities (mentioned in Table 1) of Spegazzini's and other collectors' vascular plant collections that contributed to his herbarium. Numbers refer to the Argentine provinces: 12 = Buenos Aires; 13 = Neuquén; 14 = Río Negro; 15 = Chubut; 16 = Santa Cruz; 17 = Tierra del Fuego.

populations of the growing cities there, the burning of native trees, and the introduction of exotic species (Molfino, 1929).

Some trips were undertaken by order of either the Argentine government or commercial entities. For instance, he went to Chaco to install an alcohol factory; to Tucumán to study the sugar cane disease; to western Argentina to analyze the viticultural industry; and to northwestern Argentina to search for and study plants that produce rubber. In all these trips he looked for the opportunity to collect plants because he found that it was usually difficult to organize collecting trips that lacked direct utility. In his own words:

> "Me es disgustoso declarar que he hallado constantemente una cierta indiferencia, por no decir hostilidad, toda vez que he querido llevar a cabo algún viaje botánico que no tuviera un fin práctico inmediato, así que para prepararme debidamente al desempeño de mis funciones de botánico oficial he tenido necesidad de aprovechar toda misión económica o industrial que mis conocimientos me permitiesen desempeñar más o menos satisfactoriamente, acumulando así los materiales que ahora forman la base

Table 1. Carlos Spegazzini's vascular plant-collecting expeditions, with the names of other collectors who provided specimens for him. The dates, names, and description of the travels are taken from herbarium labels and from the literature cited. * = data taken exclusively from herbarium labels.

Dates	Collecting expeditions	Bibliography
Nov. 1880	C. Spegazzini and D. Parodi; Argentina. Prov. Buenos Aires: areas surrounding the city of Buenos Aires (e.g., Boca del Riachuelo, Palermo, Recoleta, San José de Flores, Isla Maciel, Puente Alsina, Montes del Tordillo, Montes Grandes, Montes del Real Viejo).	Spegazzini, 1880, 1914b, 1917c
Dec. 1880	C. Spegazzini; Argentina. Prov. Buenos Aires: General Lavalle, Magdalena.	Spegazzini, 1917c
Dec. 1881–Nov. 1882	C. Spegazzini in Bove's expedition; Argentina. Prov. Santa Cruz: Río Gallegos, Río Santa Cruz, Salinas, Isla Pavón, Monte León, Isla de los Baguales, Isla de los Leones, Misioneros, Cabo Vírgenes. Prov. Tierra del Fuego: estrecho de Magallanes, Isla de los Estados, Punta Porpesse, Cabo Negro, Gregory Bay (Bahía San Gregorio), N coast of the province, Ushuaia, Cabo Posesión, Cerro de los Caracoles, Bahía Sarmiento, Gente Grande Bay, Punta Anegada, Isla Isabel. Chile: Punta Arenas.	Spegazzini, 1883b, 1896, 1897b, 1901c, 1902b; Moore, 1983
1883	C. Spegazzini; Uruguay. Arroyo de San Juan, Cuareim.	Spegazzini, 1917c
Early 1883	C. Spegazzini and A. Onetto; Argentina. Prov. Santa Cruz: Río Santa Cruz region.	Spegazzini, 1883a
Mar.–Nov. 1883	C. von Gülich; Argentina. Prov. Misiones: Río Piray-Guazú, Río Yacan-Guazú, near Piray. Paraguay. Tapurucupú, Sierra de Amambay.	Spegazzini, 1883a, 1916c
July–Aug. 1883	C. Spegazzini; N Argentina. Prov. Chaco. Paraguay. Río Aquidabán, Amambay.	*
1884	F. Tonini del Furia; Argentina. Prov. Santa Cruz: area of río Santa Cruz, Lago Argentino.	Spegazzini, 1897b, 1901c, 1902b; Del Vitto et al., 1998
1885	O. Mauri; Argentina. Prov. Santa Cruz: Río Chico.	Spegazzini, 1897b
Jan. 1886	C. Spegazzini; Argentina. Prov. Chaco: Colonia Resistencia, Monte Yponá.	Spegazzini, 1917c
1887	C. Spegazzini; Argentina. Prov. Buenos Aires: isla Santiago.	Spegazzini, 1905
1889	C. Moyano; Argentina. Prov. Chubut: Río Carrenleufú, Teka -choique, Río Senguer, Lago Fontana, Río Chubut, Lago Musters, Colonia Galesa.	Spegazzini, 1897c, 1901c
1889	E. Fischer; Argentina. Prov. Chubut: Cabo Raso, Puerto Rawson, Península de Valdez.	Spegazzini, 1897c; Hosseus, 1915
Dec. 1889	C. Spegazzini; Argentina. Prov. Buenos Aires: sierras Cura Malal.	*
1890	O. Mauri; Argentina. Prov. Tierra del Fuego: Canal Ultima Esperanza.	*
May–June 1890	A. Tonnellier; Argentina. Prov. Chubut: Trelew, Rawson.	*
1891	C. Spegazzini; Argentina. Prov. Buenos Aires: San Nicolás.	Spegazzini, 1905
1892	C. Spegazzini; Argentina. Prov. Buenos Aires: Tandil.	*
Jan. 1894	C. Spegazzini; Argentina. Prov. Salta: La Viña.	*
Mar. 1894	C. Ameghino; Argentina. Prov. Santa Cruz: Río Deseado, San Julián, Cañadón 11 de Septiembre, Río Salado.	Spegazzini, 1897b, 1901c 1902b
Sep. 1894	C. Berg; Argentina. Prov. Río Negro.	Spegazzini, 1901c
Jan.–Mar. 1895	C. Spegazzini; Argentina. Prov. Tucumán: Famaillá.	Spegazzini, 1895a, 1923b
Nov. 1895	C. Spegazzini; Argentina. Prov. Buenos Aires: Sierra de la Ventana in valle de las Vertientes, between Tornquist and Sierra de la Ventana, Cerro de la Ventana.	Spegazzini, 1897a, 1905
Dec. 1895	E. Fischer; Argentina. Prov. Chubut.	Spegazzini, 1902b
Late 1895–Early 1896	C. Spegazzini and S. Venturi; Argentina. Prov. Salta: Cuesta de Trancas.	Venturi, 1925

Table 1. Continued.

Dates	Collecting expeditions	Bibliography
Late 1895–Feb. 1896	C. Ameghino; Argentina. Prov. Santa Cruz: Golfo San Jorge, San Julián.	Spegazzini, 1897b, 1901c
Mar. 1896	J. Koslowski; Argentina. Prov. Chubut: Lago Fontana.	Del Vitto et al., 1998
Apr. 1896	N. Alboff; Argentina. Prov. Tierra del Fuego: San Sebastián.	Del Vitto et al., 1998
Nov. 1896	C. Spegazzini; Argentina. Prov. Buenos Aires: Tornquist, Sierra de la Ventana, Río de La Plata, Isla Santiago.	Spegazzini, 1901c, 1905
Nov. 1896–Mar. 1897	O. Mauri; Argentina. Prov. Neuquén: Río Aluminé-Neuquén.	Spegazzini, 1902c; Del Vitto et al., 1998
Late 1896–Early 1897	E. Fischer; Argentina. Prov. Chubut: Cabo Raso.	Spegazzini, 1901c, 1902b, c
Dec. 1896–Mar. 1897	C. Spegazzini; Argentina. Prov. Salta: Cuesta de Arca-Trancas, Pampa Grande, Quebrada de Guachipas, Amblaio-Cachi, Cafayate, Molinos-Cafayate, Nevado de Cachi, La Viña, cuesta de San Antonio, Isonza-Tintín, entre Talapampa y Valle Calchaquí, Runi-Huasi. Prov. Tucumán: Montero, Colalao.	Spegazzini, 1897d, 1899b, 1901d, 1916c, 1917a, b, c, d, 1921a, 1923c, 1925b
Jan.–Mar. 1897	C. Ameghino; Argentina. Prov. Santa Cruz: Río Chico (Chonquen-aik, Emelk- aik), Lago Argentino (Karr-aik).	Spegazzini, 1901c, 1902c
Feb. 1897	C. Spegazzini; Chile. Prov. Atacama: Atacama desert.	*
1897	C. Spegazzini; Argentina. Prov. Buenos Aires.	Spegazzini, 1901b, c
1897	C. Spegazzini; Argentina. Prov. Catamarca, Prov. Córdoba, Prov. La Rioja, Prov. Mendoza, Prov. Salta, Prov. San Juan.	Spegazzini, 1898
Oct. 1897	E. B., O. B., O. R., V. B. (sic); Argentina. Prov. Santa Cruz: Río Santa Cruz, Monte León.	Spegazzini, 1902b
Oct.–Nov. 1897	J. Valentín; Argentina. Prov. Chubut: Trelew, Cabo Raso, Río Chubut.	Spegazzini, 1899a, 1901c, 1902b
Nov. 1897–Jan. 1898	O. Mauri; Argentina. Prov. Neuquén: Lago Traful, Nahuel Huapí. Prov. Santa Cruz: Río Seco, Canal Ultima Esperanza.	Spegazzini, 1901c, 1902a
Oct.–Dec. 1897	C. Ameghino; Argentina. Prov. Santa Cruz: Pan de Azúcar, Río Chico (Emel-kaik, El Paso), Laguna Seca, Río Santa Cruz.	Spegazzini, 1901c, 1902b, c
Dec. 1897–Feb. 1898	C. Spegazzini; Argentina. Prov. Buenos Aires: from S Buenos Aires N Patagonia, San Blas, Carmen de Patagones, La Pantanosa, Punta Rubia, Bahía San Blas, El Carbón, Lomas de Saladero, Isla de Crespo, Salitral Grande, Salina de Piedras, Salina del Inglés, Tres Cerros, La Verde, Barrancoso. Prov. Neuquén: Lago Nahuel Huapí, Lago Traful, Laguna Blanca. Prov. Río Negro: areas of Río Colorado and Río Negro, Salina de Piedras, Puerto Nuevo, confluence Río Limay and Río Neuquén, Choele Choel, Lomas Negras.	Spegazzini, 1899a, 1901c, 1902b, c, 1905, 1914a, 1925b
1898	C. Moyano; Argentina. Prov. Río Negro.	*
Jan. 1898	C. Spegazzini; Argentina. Prov. Buenos Aires: sierras de Tandil.	Spegazzini, 1901a
Jan. 1898	F. Lahille; Argentina. Prov. Chubut: Península de Valdéz, Caleta Porfirio.	Spegazzini, 1899a, 1901c
Jan.–Apr. 1898	C. Ameghino; Argentina. Prov. Santa Cruz: Río Chico (Kumen-aik, Chonkenk-aik, cerro Kmanaich, Emelk-aik, Boron-aik, Parr-aik, Sehuen-aik), Lago Viedma (Orr-aik), Lago Argentino (Karr-aik), Río Sehuén (Parr-aik), Golfo San Jorge, Río Santa Cruz.	Spegazzini, 1899a, 1901c 1902b, c
Feb.–Mar. 1898	S. Venturi; Argentina. Prov. Santa Cruz: Río Santa Cruz.	Spegazzini, 1902b
Fall 1898	C. Ameghino; Argentina. Prov. Santa Cruz: between San Julián and Río Deseado.	Spegazzini, 1902b

Table 1. Continued.

Dates	Collecting expeditions	Bibliography
Nov. 1898–Early 1899	J. Koslowsky; Argentina. Prov. Chubut: Lago Blanco, central area of the province, Paso de los Indios, Río Senguerr, Valle del Río Mayo, Río Chubut.	Spegazzini, 1899a, 1901c 1902b
Nov. 1898–Feb. 1899	N. Illín; Argentina. Prov. Chubut: Lago Musters, Choique Lauquen, Angostura, between Trelew and Paso de los Indios, Costa de los Manantiales.	Spegazzini, 1901c, 1902b
Nov. 1898–Mar. 1899	T. Stuckert; Argentina. Prov. Córdoba.	Spegazzini, 1899b
Dec. 1898–Mar. 1899 (Spring?)	C. Ameghino; Argentina. Prov. Chubut: Lago Musters. Prov. Santa Cruz: Lago Argentino (Karr-aike), San Julián-Río Deseado, San Jorge, Lago Buenos Aires, Golfo San Jorge, Río Chico.	Spegazzini, 1902b, c
Jan., Aug. 1899	A. Tonnellier; Argentina. Prov. Chubut: Trelew, Rawson, Río Chubut.	Spegazzini, 1901c, 1902b
Apr.–May 1899	F. Lahille; Argentina. Prov. Río Negro: Golfo San Matías. Prov. Chubut: Caleta Porfirio.	Spegazzini, 1901c, 1902b
Nov. 1899–Mar. 1900	N. Illín; Argentina. Prov. Río Negro: Bolsón. Prov. Chubut: Río Chubut, between Choique-Laven and Lago Musters, Lago Musters, Teka-dique, Carrenleufú, Teka-choique.	Spegazzini, 1902a, b, c
Summer 1899	C. Moyano; Argentina. Prov. Chubut: Carrenleufú.	Spegazzini, 1902c
Dec. 1899	C. Spegazzini; Argentina. Prov. Buenos Aires: Sierras de Cura Malal, Tornquist.	Spegazzini, 1905
Late 1899–Early 1900	A. Larguía; Argentina. Prov. Río Negro: Colonia Valcheta.	Spegazzini, 1902b
Late 1899–Early 1900	F. Basaldúa; Argentina. Prov. Chubut: Trelew, Río Chubut.	Spegazzini, 1902b
Dec. 1899–Jan. 1900	C. Burmeister; Chile. Río Aisén.	Spegazzini, 1902b, c
Dec. 1899–Mar., June 1900	O. Asp; Argentina. Prov. Neuquén: Sierra de Maichol, Valle Trolope, Río Manzano, Codihué, Pilahuincó, Río Fiero, Vega del Pino Hachado, Sierras de Sanquil.	Spegazzini, 1902b
Jan.–Feb. 1900	R. Hauthal; Argentina. Prov. Santa Cruz: Cerro de los Baguales.	Spegazzini, 1902b, c
Feb. 1900	F. Silvestri; Argentina. Prov. Santa Cruz: Río Santa Cruz, Lago Argentino.	Spegazzini, 1902b, c
Feb.–Mar. 1900	N. Illín; Argentina. Prov. Chubut: Río Carrenleufú, between Cholila and Colonia 16 de Octubre.	Spegazzini, 1902b
Fall, Summer 1900	C. Ameghino; Argentina. Prov. Chubut: Col-huapí (Colhué-huapí). Prov. Santa Cruz: Río Chico, between Río Deseado and San Julián.	Spegazzini, 1902b, c
Nov. 1900	R. Hauthal; Argentina. Prov. Santa Cruz: Sierra de los Baguales.	*
Summer 1900–1901	F. Basaldúa; Argentina. Prov. Chubut: Trelew.	*
Dec. 1900	C. Burmeister; Argentina. Prov. Chubut: Arroyo Verde.	Spegazzini, 1902c
Dec. 1900	A. Fernández; Argentina. Prov. Neuquén: Lago Nahuel Huapí.	Spegazzini, 1902b, c
Dec. 1900–Feb. 1901	N. Illín; Argentina. Prov. Chubut: Lago Blanco, Corcovado, Puerto Rawson, Carrenleufú, Teka Choique, Nafofo-Cahuellu, Cholila, Manantiales.	Spegazzini, 1902b
Jan. 1901	C. Spegazzini; Argentina. Prov. Buenos Aires: Tandil.	Spegazzini, 1901a
Jan. 1901	F. Claren; Argentina. Prov. Jujuy: Puna of Santa Catalina.	*
Jan.–Apr. 1901	C. Burmeister; Argentina. Prov. Chubut: Manantial de la Subida. Chile. Río Aisén.	Spegazzini, 1902c
Feb.–Mar. 1901	C. Spegazzini; Argentina. Prov. Mendoza: Punta de Vacas, road Mendoza- Villavicencio, road Puente del Inca, Laguna de Los Horcones, valle del Aconcagua, Cerro Leones, Las Cuevas, Cañadón de los Horquillones.	Spegazzini, 1901d, 1917a, b, c, 1925a
Summer 1901	F. Lahille; Argentina. Prov. Neuquén: Fortín Roca.	Spegazzini, 1902b
Jan. 1902	R. Hauthal; Argentina. Prov. Chubut: Río Senguerr.	Del Vitto et al., 1998
Aug. 1902	C. Spegazzini; Argentina. Prov. Chaco: Ipaguayo.	*
Jan. 1903	C. Spegazzini; Argentina. Prov. Salta: Río Pescado.	Spegazzini, 1916c

Table 1. Continued.

Dates	Collecting expeditions	Bibliography
1904	C. Spegazzini; Argentina. Prov. San Juan.	Kiesling, 1994
Dec. 1904–Mar. 1905	C. Spegazzini; Argentina. Prov. Jujuy: Sierra de Santa Bárbara, Sierra de Calilegua. Prov. Salta: Río Pescado, Río San Francisco (Ledesma), Río Bermejo.	Spegazzini, 1916c, 1917a, 1921a, 1923c
Dec. 1905–Apr. 1906	C. Spegazzini; Argentina. Prov. Jujuy: Río San Francisco, Perico, Ledesma, Yuto, Río Saucelito. Prov. Salta: Orán, Valle de Lerma, Sierra de Santa Bárbara, Santa Cornelia, Sierra de Maíz Gordo, Sierra de Zenta, Río Santa María. Prov. Tucumán: Tafí, Parque Roca. Prov. Mendoza: cerro de los Cordobeses.	Spegazzini, 1914a, c, 1917c, 1923c, 1925a, c
Dec. 1905	C. Burmeister; Argentina. Prov. Santa Cruz: Puerto Deseado.	*
Dec. 1906–Mar. 1907	C. Spegazzini; Argentina. Prov. Misiones: from Santa Ana to Barracón and Río San Antonio, Puerto Esperanza (Río Paraná), Posadas, Campina de Américo, San Pedro, Fracrán, Yacán-Guazú, Garupá, Arroyo Dorado, Campo de las Cuyas, Cerro Pesegueiro, Campo Grande, Cerro Bonito, Río Bossetti (Río Grande), Río Acaranguay (Campo Grande), Loreto, Layado.	Spegazzini, 1909, 1916c, 1917a, b, c, d, 1923c, 1925b
Feb. 1907?	C. Spegazzini; Argentina. Prov. Catamarca: Piedra Blanca.	Spegazzini, 1917b
Late 1907	P. Spegazzini; Argentina. Prov. Buenos Aires: isla Martín García.	*
Dec. 1909	J. Argerich; Argentina. Prov. Buenos Aires: Estación Argerich, La Fina.	*
Dec. 1909–Mar. 1910	P. Spegazzini; Argentina. Prov. Catamarca: Ancasti, Ambato, Andalgalá, Huillapina, Pomán, Río Tala. Prov. Mendoza: quebrada de los Horcones, Cacheuta. Prov. Salta: Santa Cornelia, Sierra de Santa Bárbara. Prov. Tucumán: Alpachiri, Cochuma.	Spegazzini, 1916b, 1917c, 1923a
Mar. 1911	C. Spegazzini; Argentina. Prov. Entre Ríos: Ibicuy.	Spegazzini, 1917a
Nov. 1911	C. Spegazzini; Argentina. Prov. Jujuy: Santa Cornelia, Sierra de Santa Bárbara, Lecherón Negro.	*
1912	C. Spegazzini; Argentina. Prov. San Juan.	Kiesling, 1994
Jan.–Feb. 1914	C. Spegazzini; Argentina. Prov. Mendoza: Río Blanco del Plata. Prov. San Juan: Paso de los Puntanos.	Spegazzini, 1914a
June, Oct. 1919, Feb. 1920	C. Spegazzini; Paraguay. Asunción, Puerto Sajonia, Río Paraguay, San Antonio, Pacú-cuá, road San Lorenzo–San Antonio.	Spegazzini, 1921a, b, 1923a, c
Feb. 1922	J. Molfino; Argentina. Prov. Misiones: San Javier, Apóstoles.	*
Summer 1923	C. Spegazzini; Argentina. Prov. Buenos Aires: Isla Marín García.	Anonymous, 1925
Jan. 1924	C. Spegazzini; Argentina. Prov. Tierra del Fuego: Haberton (Shamanes), Isla Grande, Lapataia, Ushuaia, Bahía Orange, Puerto Shell. Chile. Cabo de Hornos, Isla Hoste, Península Hardy.	Spegazzini, 1924; Moore, 1983
Jan. 1926	A. Raffaelli; Argentina. Prov. Río Negro: S Río Negro, Talagapa, Barrilniyeo, Carilaufquen.	Spegazzini, 1926

del grandioso Herbario Argentino que figura en la Sección Botánica Ministerio de Agricultura y el conocimiento que tengo de la fito-geografía argentina"(Spegazzini, 1909). [I am annoyed to declare that I have constantly found a certain indifference, if not hostility, every time I have wanted to make a botanical expedition devoid of immediate profit. Thus to properly prepare myself to fulfill my official botanical duties I have had to take advantage of every economic or industrial mission that my knowledge allowed me to do more or less satisfactorily, accumulating in this way the materials that now constitute the basis of the great Herbario Argentino of the Sección Botánica Ministerio de Agricultura and the knowledge that I have about Argentine phytogeography.]

His partners on the field trips described Spegazzini as a true naturalist, an enthusiastic and profound connoisseur of the flora and fauna, which he described in simple language, making each expedition an exciting and enjoyable adventure (Molfino, 1929; Parodi, 1961).

Spegazzini's Specimens

As a result of the collecting expeditions made by Carlos Spegazzini and by his collaborators, the number of vascular plants in Spegazzini's herbarium reached 100,000 specimens (Molfino, 1929). This number is not surprising if one bears in mind that he was able to collect 30,000 specimens in a single trip (Venturi, 1925). Due to the abundance of fungi and vascular plants collected, Spegazzini maintained the majority of them as a personal herbarium at home. Several European and North American institutions were interested in purchasing these valuable collections after Spegazzini's death, but all offers were rejected. Around 1966 (Kiesling, 1984), the collection of vascular plants was transfered from LPS to the Herbarium of Museo de La Plata (LP), also in La Plata city.

Type materials ascribed to Spegazzini are estimated at ca. 700 specimens in LP. Staff of this herbarium (Katinas et al., in prep.) are currently developing a catalogue of these specimens. Other specimens remain in Museo de Botánica Juan A. Domínguez in Buenos Aires (BAF), in the Instituto de Recursos Biológicos (INTA) in Buenos Aires (BAB), as well as in the Museo Botánico in Córdoba (CORD). Other herbaria with Spegazzini vascular plant specimens are: BAE, BR, E, H, IAC, K, L, MICH, NY, PAC, PAD, S, U, W, and Z (Stafleu & Cowan, 1985).

Two additional Spegazzini collections of interest are, or were, the living types of cacti, and the photographs of cacti. The collection of living types is practically lost, except for one specimen of *Cereus* still growing in his house, now the institute (Kiesling, 1984). His collection of cacti photographs that correspond to type and non-type specimens (Kiesling, 1984) is deposited in the Herbarium of Museo de La Plata (LP).

Specimen labels are annotated by Spegazzini or stamped with a seal by him, with a few references to the locality. He used the initials C. S. for the plants he collected, and other initials to differentiate other collectors: C. A. (for Carlos Ameghino), N. I. (for Nicolás Illín), T. F. (for Tonini del Furia). Most of the specimens lack the number of the individual collector, although they have the number of Spegazzini's herbarium (LPS). For example, the type specimen of *Oryzopsis bicolor* (Vahl) Speg. var. *media* Speg. collected by Carlos Spegazzini in 1899 should be cited: *C. Spegazzini s.n.* (ex LPS 12517 in LP). The name of the collector is frequently not mentioned, but can usually be determined by reference to the year and locality (Table 1). The date of collection usually consists of the month and the year, or sometimes the year is accompanied by the terms *aest.* (from the Latin aestivus or summer) or *aut.* (autumnus, fall). The word *vere*, annotated by Spegazzini as "Vere 1889," can be seen only in the labels of the specimens collected by Carlos Ameghino in San Julián-Río Deseado. There are two probable meanings for this Latin term (Stearn, 1996): (1) *Ver* = spring, a neuter generic noun of the third declension. *Vere* corresponds to the ablative singular, i.e., "by the spring," "with the spring," or "from the spring"; and (2) *Vere* (also *vero, revera*) = an adverb meaning "truly, in fact, rightly, exactly." Following other annotations of Spegazzini as "Vere 1894" (Spegazzini, 1895b) for fungi collected in October–November (Southern Hemisphere spring), it seems that the most reasonable interpretation of this term is "spring."

For some labels of Patagonian specimens, there is no citation of the contemporary political provinces. From 1878 to 1884 the Argentine territory south of Río Colorado (ca. 40°S latitude) to Cabo de Hornos was established by the government as one unit: Gobernación de la Patagonia. Only in 1884 was this wide territory divided into the current provinces of La Pampa, Neuquén, Río Negro, Chubut, Santa Cruz, and Tierra del Fuego. Another problem with some of these Patagonian plants is with the specimens collected by C. Moyano in Chubut in 1899. As Moyano did not write the corresponding labels, Spegazzini annotated the labels with probable localities. As he explained (Spegazzini, 1897c):

> "La colección que voy a publicar se hallaba en bastante buen estado de conservación a pesar de no haber sido envenenada, pero por desgracia carecía completamente de etiquetas, de modo que los habitat he debido indicarlos de un modo bastante vago y por recuerdos más o menos del coleccionador." [The collection that I am going to publish was in a quite good state of conservation although it was not curated with preservative. Unfortunately, it completely lacked labels, so I had to indicate the habitat very vaguely and more or less by the recollections of the collector.]

The type specimens frequently have the original Latin descriptions made in Spegazzini's handwriting. Many of these descriptions were written directly in the field or during the specimen preparation (Fig. 4). It is also very common to find the

Figure 4. Original description of a new variety of vascular plant made in Spegazzini's handwriting.

botanist's accompanying drawings, characterized by their precision and simplicity (Fig. 5). Some sets of the original Spegazzini illustrations are housed in BAF, as is the case with some cactus species (Kiesling, 1984). The citation of more than one specimen and locality in the original taxa descriptions (species, varieties, and forms) is common, and therefore Spegazzini's type collection is mainly

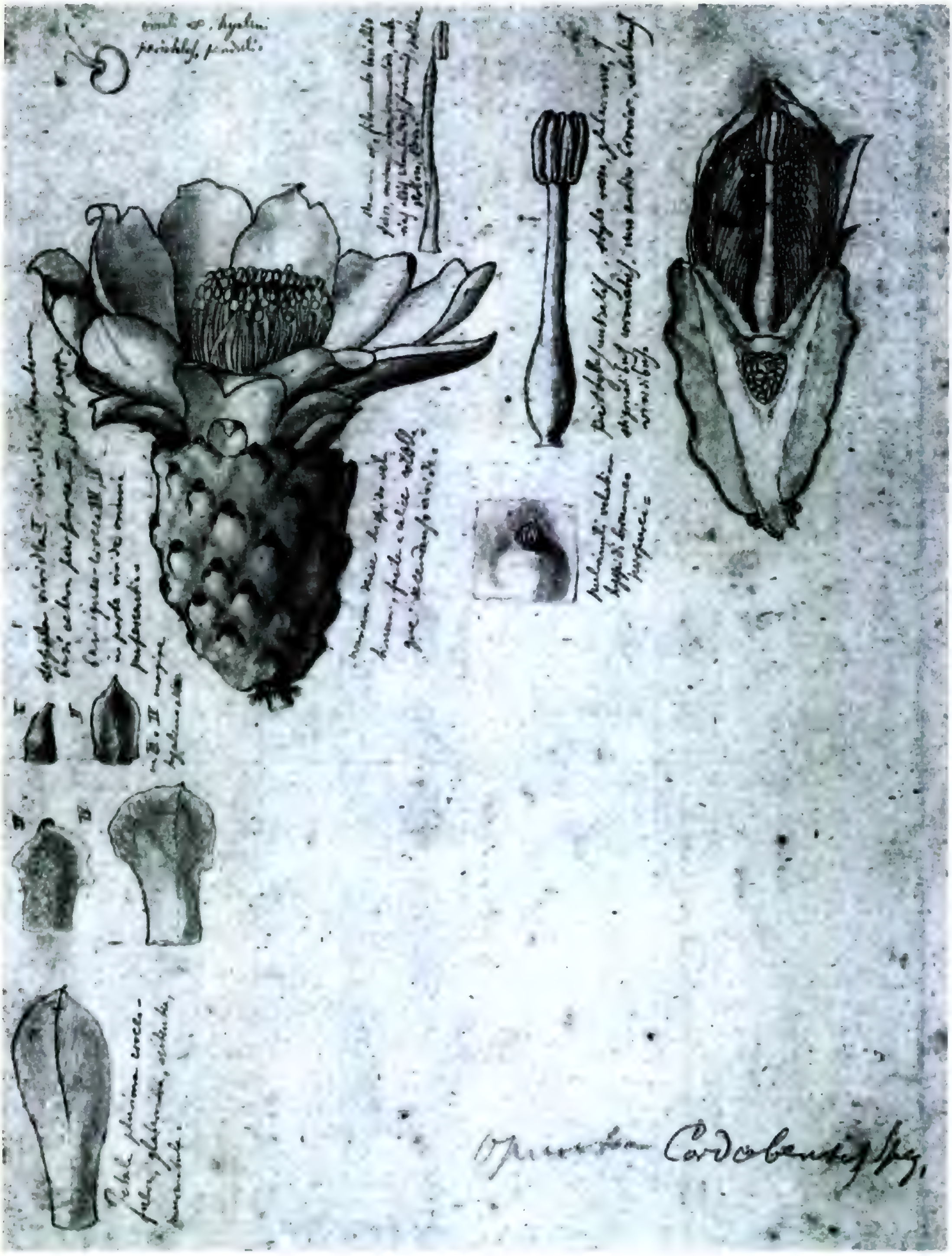

Figure 5. Accompanying drawing of a plant specimen made by Spegazzini.

made up of syntypes. Types are housed in a separate collection in LP, but it is probable that some types still remain in the general herbarium. The search for these specimens is another step in the production of the catalogue mentioned above.

Spegazzini's Publications

Carlos Spegazzini published 332 papers from 1878 to 1926, and ca. 100 refer to vascular plants. Almost all of them were published in such Argen-

Table 2. New taxa in their corresponding families described by Carlos Spegazzini, as cited in *Index Kewensis* (Anonymous, 1997), and the Gray Card Index Database. The number of taxa described in each taxonomic category is in parentheses. Spegazzini is the authority of all names except where marked. The designation of comb. illeg., comb. nov., comb. superfl., hom. illeg., nom. illeg., nom. inval., nom. nud., and a change in the authority of the name other than Spegazzini (marked with *) follows Zuloaga et al. (1994) and Zuloaga & Morrone (1996, 1999a, b).

AIZOACEAE. Species (1): *Tetragonia ameghinoi.* Total (1).

ALISMATACEAE. Species (1): *Echinodorus patagonicus.* Total (1).

AMARANTHACEAE. Genera (1): *Amarantellus.* Species (4): *Amarantellus argentinus, Amaranthus cristulatus, A. vulgatissimus, Amarantus edulis.* Infraspecific taxa (1): *Blutaparon portulacoides* var. *commersonii**. Total (6).

AMARYLLIDACEAE. Species (3): *Zephyranthes lilacina, Z. melanopotamica, Z. oxitepala.* Infraspecific taxa (2): *Hippeastrum bagnoldi* var. *minor, H. hesperius* var. *pallida.* Total (5).

ANACARDIACEAE. Species (4): *Lithrea chichita**, *Schinus chichita, S. longifolia* (Lindl.) Speg. comb. nov., *S. praecox.* Infraspecific taxa (1): *Schinus dependens* var. *patagonica.* Total (5).

APIACEAE. Genera (1): *Notiosciadium.* Species (13): *Asteriscium fimbriatum, Azorella ameghinoi, A. bovei, A. fuegiana, A. patagonica, A. plantaginea, Hydrocotyle cryptocarpa, Mulinum lycopodioides, M. morenonis* (Kuntze) Speg. comb. nov., *M. patagonicum, M. valentinii, Notiosciadium pampicola, Sanicula patagonica.* Infraspecific taxa (4): *Azorella patagonica* var. *compacta, Bowlesia tropaeolifolia* var. *heterophylla, B. tropaeolifolia* var. *patagonica, Hydrocotyle araucana* var. *patagonica.* Total (18).

APOCYNACEAE. Species (5): *Aspidosperma chakensis, A. crotalorum, A. horco-kebracho, A. missionum, Rauvolfia schuelii.* Infraspecific taxa (1): *Aspidosperma quebracho-blanco* var. *pendula.* Total (6).

AQUIFOLIACEAE. Species (1): *Ilex tucumanensis* nom. nud. Total (1).

ARACEAE. Genera (1): *Lilloa.* Species (2): *Lilloa puki, Staurostigma vermicida.* Total (3).

ARECACEAE. Species (2): *Maximiliana argentinensis, M. orenocensis.* Total (2).

ARISTOLOCHIACEAE. Species (2): *Aristolochia melanoglossa, A. stuckertii.* Total (2).

ASCLEPIADACEAE. Genera (1): *Dicarpophora.* Species (4): *Astephanus fruticulosus, Dicarpophora mazzuchii, Oxypetalum suaveolens* hom. illeg., *Vincetoxicum bulligerum.* Infraspecific taxa (1): *Philibertia gilliesii* var. *pubescens.* Total (6).

ASTERACEAE. Genera (2): *Ameghinoa, Strongylomopsis.* Species (58): *Achyrophorus leucanthus, Ameghinoa patagonica, Aplopappus (= Haplopappus?) moyanoi, A. mustersi, A. patagonicus, A. struthionum, A. tehuelches, Aster scorzonerifolius, Baccharis chubutensis, B. melanopotamica, B. tandilensis, Brachyclados caespitosus* (Phil.) Speg. comb. nov., *B. megalanthus, B. stuckertii, Chuquiraga argentea* (Speg.) Speg. comb. nov., *Culcitium gilliesii* (Hook. & Arn.) Speg. comb. nov., *C. sessile, Doniophyton argenteum, Erigeron erianthus, E. platensis, E. remyanus**, *Flotovia stiffioides, Gutierrezia ameghinoi, Haplopappus ameghinoi, H. illinii, H. moyanoi, H. mustersii, H. patagonicus, H. struthionum, H. tehuelches, Hieracium chubutense, Leuceria eriocephala, L. patagonica, Mutisia chubutensis, M. moyanoi, M. pulchella, Nassauvia ameghinoi, N. chubutensis, N. patagonica, N. pentacaenoides, Perezia megalantha, P. pampeana, P. patagonica, P. sessiliflora, Psilocarphus globiferus, Senecio argentinensis, S. capillarifolius, S. choiquelahuensis, S. chubutensis, S. colu-huapensis, S. diabolicus, S. inutilis, S. julianus, S. mustersii, S. sagittarioides, S. sericeo-nitens, Strongylomopsis fuegiana, Vernonia oreophila.* Infraspecific taxa (37): *Baccharis trimera* var. *viscosissima, Chiliotrichum diffusum* var. *angustifolia, Chiliotrichum diffusum* var. *media, Chiliotrichum diffusum* var. *typica, Chuquiraga argentea* var. *dusenii, Erigeron gayanus* var. *leptophyllus, E. philippii* var. *elatior, E. philippii* var. *humilis, Gnaphalium affine* var. *medium, G. affine* var. *parvulum, G. affine* var. *pusillum, G. purpureum* var. *sphacelatum* (Kunth) Speg. comb. nov., *Grindelia speciosa* var. *integrifolia, Gutierrezia paniculata* var. *patagonica, Hieracium antarcticum* f. *fuegiensis, Hypochaeris arenaria* var. *coronopifolia* (Sch. Bip.) Speg. comb. nov., *H. arenaria* var. *integrifolia* (Sch. Bip.) Speg. comb. nov., *H. variegata* var. *acutibracteata, H. variegata* var. *glaucescens, H. variegata* var. *nana, H. variegata* var. *typica, H. variegata* var. *pinnatifida, Leuceria ibari* var. *glabrata, L. ibari* var. *glandulosa, L. ibari* var. *sessiliflora, Nassauvia abbreviata* var. *azorelloides, N. axillaris* var. *contracta, N. patagonica* var. *elatior, N. struthionum* var. *robusta, Panargyrum abbreviatum* var. *subspinosa, Perezia patagonica* var. *intermedia, Senecio desideratus* f. *elatiuscula, S. linariifolius* var. *angustissimus* (Phil.) Speg. comb. nov., *S. miser* var. *tehuelches, S. trifurcatus* var. *pentadactylus* (Phil.) Speg. comb. nov., *S. xanthoxylon* var. *araneosula, Solidago linearifolia* var. *brachypoda.* Total (97).

BEGONIACEAE. Species (1): *Begonia argentinensis.* Total (1).

BIGNONIACEAE. Species (1): *Tecoma avellanedae.* Infraspecific taxa (1): *Argylia potentillaefolia* var. *australis.* Total (2).

BORAGINACEAE. Genera (3): *Oxyosmyles, Valentina, Valentiniella.* Species (10): *Amsinckia patagonica, A. pseudolycopsicoides, Echinospermum patagonicum, Eritrichium diffusum* hom. illeg., *E. mesembryanthemoides, E. pampeanum, Heliotropium lithospermifolium, Oxyosmyles viscosissima, Valentina patagonica, Valentiniella patagonica* (Speg.) Speg. comb. nov. Infraspecific taxa (1): *Amsinckia angustifolia* var. *microcarpa.* Total (14).

Table 2. Continued.

BRASSICACEAE. Genera (2): *Delpinoella, Delpinophytum.* Species (40): *Braya cachensis, B. glebaria, B. lycopodioides, B. patagonica, B. pectinata, B. pycnophylloides, Cardamine argentina, C. callitrichoides, C. patagonica, Delpinoella patagonica, Delpinophytum patagonicum* (Speg.) Speg. comb. nov., *Descurainia deserticola* (Speg.) Speg. comb. nov., *D. glabrescens* (Speg.) Speg. comb. nov., *D. heterotricha, Draba ameghinoi, D. argentina, D. chubutensis, D. glebaria* Speg. ex O. E. Schulz, *D. graminifolia, D. karr-aikensis, D. oligosperma, D. pectinata* Speg. ex O. E. Schulz, *Menonvillea patagonica, Nasturtium pamparum, N. philippianum* nom. illeg., *N. platense, Schizopetalum fuegianum, Sisymbrium ameghinoi, S. andinum* hom. illeg., *S. deserticola, S. fuegianum* (Speg.) Speg. comb. nov., *S. glabrescens, S. glanduliferum, S. maclovianum, S. patagonicum, S. perenne* hom. illeg., *S. pinnatum, S. subscandens, S. tehuelches, Thlaspi chionophilum.* Infraspecific taxa (21): *Braya lycopodioides* var. *contracta, Cardamine rostrata* var. *dichondroides, C. tuberosa* var. *velutina, Descurainia canescens* var. *patagonica, D. canescens* var. *purpureola, Draba argentina* var. *grandiflora, D. argentina* var. *latifolia, D. karr-aikensis* var. *major, D. karr-aikensis* var. *media, D. karr-aikensis* var. *minor, D. magellanica* var. *glabrata* Gilg. ex Speg., *D. magellanica* var. *subglabrata, Lepidium pubescens* var. *salinicola, L. pubescens* var. *typica, Sisymbrium fuegianum* var. *glabrum, S. fuegianum* var. *hispidum, S. sagittatum* var. *commune, S. sagittatum* var. *exauriculatum, S. sagittatum* var. *glaucum, S. sagittatum* var. *normalis, S. sagittatum* var. *purpurascens.* Total (63).

BROMELIACEAE. Species (6): *Aechmea thyrsigera, Puya flora, P. formosa, Tillandsia chlorantha, T. euosma, Vriesea argentinensis.* Infraspecific taxa (1): *Dyckia montevidensis* var. *tandilensis.* Total (7).

CACTACEAE. Genera (4): *Aylostera, Brittonrosea, Maihueniopsis, Parodia.* Species (113): *Austrocactus dusenii*, A. intertextus, Aylostera pseudominuscula* (Speg.) Speg. comb. nov., *Brittonrosea albata, B. anfractuosa, B. arrigens, B. confusa, B. coptonogona, B. crispata, B. dichroacantha, B. gladiata, B. grandicornis, B. hastata, B. heteracantha, B. lamellosa, B. lancifera, B. lloydii, B. multicostata, B. obvallata, B. pentacantha, B. phyllacantha, B. violaciflora, B. wippermanni, B. zacatecasensis, Cereus dayami, C. dusenii*, C. guelichii, C. patagonicus*, C. platygonus* hom. illeg., *C. roseiflorus, C. santiaguensis, C. silvestrii, C. smaragdiflorus*, C. thelegonoides, Echinocactus arechavaletai, E. baldianus, E. cachensis, E. caespitosus, E. catamarcensis* hom. illeg., *E. chionanthum, E. famatinensis, E. haematanthus, E. loricatus* hom. illeg., *E. pampeanus, E. parvulum, E. platensis, E. pseudominusculus, E. pygmaeus, E. saltensis, E. sanjuanensis, E. stellatus, E. stuckertii, E. thionanthum, Echinopsis ancistrophora, E. baldiana, E. cachensis, E. cordobensis, E. intricatissima, E. melanopotamica, E. minuana, E. mirabilis, E. molesta, E. pseudominuscula, E. saltensis, E. silvestrii, E. spegazzinii* K. Schum. ex Speg.*, *Frailea bruchii, F. odieri, F. pulcherrima, Gymnocalycium baldianum* (Speg.) Speg. comb. nov., *G. brachypetalum, G. chubutense* (Speg.) Speg. comb. nov., *G. leptanthum, G. loricatum, G. parvulum* (Speg.) Speg. comb. nov., *G. stellatum, Leocereus paulensis, Lobivia areopegon* Speg. ex Hosseus, *L. hyalacantha, L. oreopepon, L. shaferi*, Maihuenia tehuelches, M. valentinii, Maihueniopsis molfinoi, Malacocarpus sanjuanensis, Opuntia anacantha, O. arechavaletae, O. atro-virens, O. bonarensis, O. bruchii, O. canina, O. chaquensis, O. cordobensis, O. halophila, O. hypsophila, O. kiska-loro, O. molinensis, O. montevidensis, O. pampeana, O. penicilligera, O. prasina, O. retrorsa, O. schumannii, O. subsphaerocarpha, O. tuna-blanca, O. utkilio, O. weberi, Parodia brasiliensis, P. microsperma* (F. A. C. Weber) Speg. comb. nov., *P. paraguayensis, Pilocereus rhodacanthus* (Salm-Dyck) Speg. comb. nov., *Pterocactus valentinii, Rebutia famatinensis* (Speg.) Speg. comb. nov., *Tephrocactus hickeni* (Britton & Rose) Speg. comb. nov. Infraspecific taxa (35): *Cereus lamprochlorus* var. *salinicola, Echinocactus acuatus* var. *corynodes, E. acuatus* var. *depressus, E. acuatus* f. *erinacea, E. acuatus* var. *sellowii, E. acuatus* var. *tetracantha, E. catamarcensis* var. *obscura, E. catamarcensis* var. *pallida, E. gibbosus* var. *cerebriformis, E. gibbosus* var. *chubutensis, E. gibbosus* var. *platensis, E. gibbosus* var. *typica, E. gibbosus* var. *ventanicola, E. mammulosus* var. *hircina, E. mammulosus* var. *pampeanus, E. mammulosus* var. *submammulosa, E. mammulosus* var. *typica, E. microspermus* var. *erythranthus, E. microspermus* var. *thionanthus, E. platensis* var. *leptanthus, E. platensis* var. *parvulus, E. platensis* var. *quehlianus, E. platensis* var. *typica, E. pygmaeus* var. *phaeodiscus, Echinopsis leucantha* var. *brasiliensis, Opuntia bruchii* f. *macracantha, O. diademata* var. *inermis, O. diademata* var. *oligacantha, O. diademata* var. *polyacantha, O. ficus-indica* var. *decumana, O. ficus-indica* var. *gymnocarpa*, Tephrocactus bruchi* f. *brachyacantha, T. bruchii* f. *macracantha, T. glomeratus* var. *inermis, T. glomeratus* var. *oligacanthus.* Total (152).

CALYCERACEAE. Species (12): *Boopis ameghinoi, B. chubutensis, B. filifolia, B. leptophylla, B. patagonica, B. raffaellii, Gamocarpha ameghinoi, G. caleofuensis, G. patagonica, G. subandina, Nastanthus chubutensis, N. patagonicus.* Infraspecific taxa (3): *Boopis crassifolia* var. *spinuligera, B. gracilis* var. *lazulina, Gamocarpha subandina* var. *glaucescens.* Total (15).

CAMPANULACEAE. Species (1): *Downingia pusilla*.* Total (1).

CAPPARACEAE. Species (1): *Cleome titubans.* Total (1).

Table 2. Continued.

CARYOPHYLLACEAE. Genera (1): *Philippiella*. Species (7): *Lychnis argentina, L. chubutensis, L. patagonica, Melandrium chubutense* (Speg.) Speg. comb. nov., *M. patagonicum* (Speg.) Speg. comb. nov., *Philippiella patagonica, Stellaria chubutensis*. Infraspecific taxa (11): *Arenaria serpens* var. *andicola**, *Arenaria serpens* var. *microphylla* (Phil.) Speg. comb. nov., *Arenaria serpens* var. *palustris* (Naudin) Speg. comb. nov., *Arenaria serpens* var. *patagonica* (Phil.) Speg. comb. nov., *Arenaria serpens* f. *robusta, Arenaria serpens* var. *serpylloides, Sagina apetala* var. *melanopotamica, S. apetala* var. *paludosa, Silene inflata* var. *patagonica, Stellaria media* var. *apetala, S. media* var. *normalis* nom. inval. Total (19).

CELASTRACEAE. Species (2): *Schaefferia argentinensis, S. uruguayensis*. Total (2).

CHENOPODIACEAE. Species (18): *Atriplex ameghinoi, A. argentina, A. espostoi, A. flavescens* hom. illeg., *A. frigida, A. macrostyla, A. mendozaensis, A. platensis, A. robusta* Speg. nom. nud., *A. sagittifolia, A. vulgatissima, Chenopodium ameghinoi, C. antarcticum* (Hook. f.) Speg. comb. superfl., *C. fuegianum, C. scabricaule, Holmbergia tweedii* (Moq.) Speg. comb. nov., *Spirostachys olivascens, Suaeda patagonica*. Infraspecific taxa (20): *Atriplex sagittifolia* var. *heterophylla, A. sagittifolia* var. *macrophylla, A. sagittifolia* var. *microphylla, A. sagittifolia* var. *typica* nom. inval., *Blitum rubrum* var. *hypoleuca, B. rubrum* var. *macrosperma* (Hook. f.) Speg. comb. nov., *Chenopodium ambrosioides* var. *oblanceolata, C. ambrosioides* var. *chilensis**, *C. ambrosioides* var. *graveolens* (Willd.) Speg. comb. nov., *C. ambrosioides* var. *oboyata, C. ambrosioides* var. *typica, C. scabricaule* f. *megalosperma, C. scabricaule* f. *pusilla, C. scabricaule* f. *robusta, Lerchea fruticosa* var. *brachyphylla, L. fruticosa* var. *megalosperma, Salicornia corticosa* var. *procumbens, S. corticosa* var. *typica* nom. inval., *S. fruticosa* var. *doeringii* (Lorentz & Niederl.) Speg. comb. nov., *S. fruticosa* var. *macrostachya*. Total (38).

CONVOLVULACEAE. Species (3): *Convolvulus platigena, C. platincola, Ipomoea argentinensis*. Total (3).

CYPERACEAE. Species (3): *Carex patagonica, C. subantarctica, Eleocharis funebris*. Infraspecific taxa (3): *Carex darwinii* var. *urolepis**, *Eleocharis acicularis* var. *lilliputiana, Uncinia phleoides* var. *brachytricha*. Total (6).

ELATINACEAE. Species (1): *Elatine nivalis*. Total (1).

ERICACEAE. Species (3): *Pernettya chubutensis, P. patagonica, P. philippiana*. Total (3).

EUCRYPHIACEAE. Species (1): *Eucryphia patagonica*. Total (1).

EUPHORBIACEAE. Genera (1): *Aonikena*. Species (9): *Aonikena patagonica, Aporosella chacoensis* (Morong) Speg. comb. nov., *Colliguaja patagonica, Croton ventanicolus, Euphorbia pampeana, E. pseudopeplus, Jatropha antisyphilitica, J. guaranitica, Phyllanthus marginivillosus*. Infraspecific taxa (1): *Euphorbia portulacoides* var. *obtusifolia**. Total (11).

FABACEAE. Genera (7): *Anadenanthera, Cavaraea, Chiovendea, Manganaroa, Pirottantha, Pithecodendron, Ramorinoa*. Species (93): *Acacia etilis, A. nitidifolia, Adesmia ameghinoi, A. aphanantha, A. canescens* (A. Gray) Speg. comb. nov., *A. graminidea, A. karraikensis, A. leptopoda, A. pampeana, A. patagonica, A. rudolfi, A. salicornioides, A. tehuelcha, Anadenanthera falcata, A. peregrina, Anarthrophyllum patagonicum, A. subandinum, Astragalus aconcaguensis, A. ameghinoi, A. benthamianus, A. bungeanus, A. chubutensis, A. grandis* (Kuntze) Speg. comb. nov., *A. hohenackeri, A. hurtadensis, A. maulensis, A. megalocarpus, A. meyenianus, A. moyanoi, A. nuttalianus, A. philippii, A. reichei, A. rhudolphii, A. sanctae-crucis, A. santiagensis, A. subandinus, A. tarapacanus, A. tehuelches, A. valparadisiensis, A. watsonianus, Calliandra brachyandra, C. grisebachiana* (Harms) Speg. comb. nov., *C. parvifolia* (Hook. f. & Arn.) Speg. comb. nov., *Cassia carnaval, Cavaraea elegans, Chiovendea eriantha, C. hypoleuca, Erythrina chacoensis* nom. nud., *Hoffmanseggia patagonica, Manganaroa alemquerensis, M. altiscandens, M. articulata, M. furcata* (Gillies ex Hook. & Arn.) Speg. comb. nov., *M. martii* (Benth.) Speg. comb. nov., *M. monacantha* (Willd.) Speg. comb. nov., *M. paniculata* (Willd.) Speg. comb. nov., *M. paraensis, M. platensis* (Manganaro) Speg. comb. nov., *M. subsericea, M. velutina, Mimosa ostenii* Speg. ex Burkart, *M. striata* (Benth.) Speg. comb. nov., *M. tandilensis, Patagonium ameghinoi, P. aphananthum, P. berteroi, P. canescens, P. carnosum* (Dusén) Speg. comb. nov., *P. filipes, P. graminideum, P. griseum, P. karraikense* (Speg.) Speg. comb. nov., *P. leptopodum, P. patagonicum, P. rudolphi, P. salicornioides* (Speg.) Speg. comb. nov., *P. serrazzianum, P. silvestrii, P. simonsi, P. tehuelches, P. triphyllum, P. vallis-pulchrae, P. villosum, Pirottantha modesta, Pithecodendron argentinensis, Prosopis patagonica, Pterocarpus valentinii, Ramorinoa girolae, Trifolium argentinense, Vachellia astringens* (Gillies ex Hook. & Arn.) Speg. comb. nov., *Vicia platensis, V. sericella, Xerocladia pampeana*. Infraspecific taxa (64): *Acacia adhaerens* var. *parviceps, A. praecox* f. *armata, A. praecox* f. *inermis, A. riparia* var. *argentinensis, Adesmia filipes* var. *obtusifolia, A. lotoides* var. *brachypoda, A. lotoides* var. *elata, A. lotoides* var. *normalis, A. lotoides* var. *petiolulata, A. lotoides* var. *typica, A. patagonica* var. *nana, Anarthrophyllum desideratum* var. *bergii* (Hieron.) Speg. comb. nov., *A. desideratum* var. *morenonis* (Kuntze) Speg. comb. nov., *A. desideratum* var. *mustersii, A. desideratum* var. *typica, A. rigidum* var. *toninii, Astragalus moyanoi* var. *villosula, A. palenae* var. *grandiflorus, A. rengifoi* var. *lejocarpa, A. sanctae* var. *crucis, Calliandra grisebachiana* var. *carolae, Erythrina crista-galli* var. *inermis* nom. nud., *Hoffmanseggia trifoliata* var. *glaberrima, H. trifoliata* var. *glandulosa, H. trifoliata* var. *microphylla, H. trifoliata* var. *normalis, H. trifoliata* var. *pentaphylla, Lathyrus cicera* var. *patagonica, L. magellanicus* var. *glaucescens, L. magellanicus* var. *oxyphylla, L. pubescens* var. *glaucescens, L. pubescens* var. *leptophylla, L. pubescens* var. *normalis, L.*

Table 2. Continued.

stipularis var. *patagonica*, *Lonchocarpus neuroscapha* var. *pubescens*, *Manganaroa paniculata* var. *paraguayensis*, *M. velutina* var. *glabrescens*, *Patagonium lanatum* var. *axillaris*, *P. lanatum* var. *parvifolia*, *P. villosum* var. *acutifolia*, *P. villosum* var. *glabratum*. *P. villosum* var. *typica*, *Vachellia farnesiana* f. *armata*, *V. farnesiana* f. *brachypoda*, *V. farnesiana* f. *cavenia* (Molina) Speg. comb. nov., *V. farnesiana* f. *inermis*, *V. farnesiana* f. *micrantha*, *V. farnesiana* f. *microcarpa*, *V. farnesiana* f. *stenocarpa*, *V. farnesiana* f. *typica*, *V. lutea* f. *aroma* (Gillies ex Hook. & Arn.) Speg. comb. nov., *V. lutea* var. *aroma*, *V. lutea* f. *leptocarpa*, *V. lutea* f. *moniliformis* (Griseb.) Speg. comb. nov., *V. lutea* f. *oocephala*, *V. lutea* f. *pachycarpa*, *V. lutea* f. *thlipsacantha*, *Vicia bijuga* var. *longipes*, *V. patagonica* var. *depauperata* (Clos) Speg. comb. nov., *V. sericella* var. *glabrata*, *V. vicina* var. *azurea*, *V. vicina* var. *luteiflora*, *V. vicina* var. *pallidiflora*, *V. vicina* var. *tricolor*. Total (164).

FLACOURTIACEAE. Species (1): *Banara glandulosa* (Desv.) Speg. comb. nov. Total (1).

FRANKENIACEAE. Species (3): *Frankenia chubutensis*, *F. pampeana*, *F. patagonica*. Infraspecific taxa (3): *Frankenia microphylla* var. *juniperinoides* (Hieron.) Speg. comb. nov., *F. microphylla* var. *relaxata*, *F. microphylla* var. *typica*. Total (6).

GENTIANACEAE. Species (1): *Erythraea ameghinoi*. Infraspecific taxa (5): *Gentiana magellanica* var. *darwinii* (Griseb.) Speg. comb. nov., *G. magellanica* var. *typica*, *G. magellanica* f. *albiflora*, *G. magellanica* f. *cyanescens*, *G. magellanica* f. *pumila*. Total (6).

GERANIACEAE. Species (1): *Geranium melanopotamicum*. Infraspecific taxa (2): *Erodium cicutarium* var. *arenicola* (Steud.) Speg. comb. nov., *Geranium dissectum* var. *patagonica*. Total (3).

HALOPHYTACEAE. Genera (1): *Halophytum*. Species (1): *Halophytum ameghinoi* (Speg.) Speg. comb. nov. Total (2).

HYDNORACEAE. Species (2): *Prosopanche bonacinae*, *P. mazzuchii*. Infraspecific taxa (2): *Prosopanche burmeisteri* var. *bettfreundii*, *P. burmeisteri* var. *minor*. Total (4).

IRIDACEAE. Species (3): *Cypella elegans*, *C. oreophila*, *Symphyostemon patagonicus*. Infraspecific taxa (1): *Sisyrinchium striatum* var. *microspathum* (Phil.) Speg. comb. nov. Total (4).

JUNCACEAE. Species (1): *Luzula patagonica*. Total (1).

JUNCAGINACEAE. Species (1): *Triglochin monanthos*. Total (1).

LAMIACEAE. Species (2): *Scutellaria platensis*, *Sphacele pampeana*. Infraspecific taxa (5): *Micromeria darwinii* var. *imbricatifolia*, *M. darwinii* var. *pallida*, *M. darwinii* var. *pusilla* (Phil.) Speg. comb. nov., *M. darwinii* var. *typica* nom. inval., *M. darwinii* var. *virescens*. Total (7).

LENTIBULARIACEAE. Species (1): *Utricularia platensis*. Total (1).

LILIACEAE. Genera (2): *Schickendantzia*, *Schickendantziella*. Species (7): *Brodiaea ameghinoi*, *B. patagonica* hom. illeg., *Schickendantzia trichosepala*, *S. pygmaea*, *Schickendantziella trichosepala* (Speg.) Speg. comb. nov., *T. eremophyllum*, *T. pulchellum*. Infraspecific taxa (2): *Brodiaea patagonica* var. *angustiloba*, *Triteleia patagonica* var. *angustiloba*. Total (11).

LOASACEAE. Species (1): *Loasa patagonica*. Infraspecific taxa (2): *Blumenbachia silvestris* var. *leptocarpa*, *Loasa pinnatifida* var. *gracilis*. Total (3).

LYTHRACEAE. Species (1): *Pleurophora patagonica*. Total (1).

MALVACEAE. Genera (1): *Lecanophora*. Species (13): *Abutilon eriocarpum* Speg. ex Stuckert nom. nud., *A. vidalii* (Phil.) Speg. comb. nov., *Cristaria kuntzei*, *C. linoides* (Hieron.) Speg. comb. nov., *Hibiscus argentinus* hom. illeg., *H. pulcherrimus*, *Lecanophora patagonica* (Kuntze) Speg. comb. nov., *Sida ameghinoi*, *S. chubutensis*, *S. linoides* (Hieron.) Speg. comb. nov., *S. tehuelches*, *Sphaeralcea australis*, *S. patagonica* (Niederl.) Speg. comb. nov. Infraspecific taxa (4): *Sphaeralcea patagonica* var. *argentea*, *S. patagonica* var. *cinerascens*, *S. patagonica* var. *normalis*, *S. patagonica* var. *oxyodonta*. Total (18).

MARTYNIACEAE. Species (1): *Craniolaria argentina*. Total (1).

MELASTOMATACEAE. Species (1): *Comolia platensis*. Total (1).

MISODENDRACEAE. Species (1): *Misodendrum patagonicum*. Total (1).

MORACEAE. Species (1): *Urostigma quintuplinerve*. Infraspecific taxa (1): *Brosimum gaudichaudii* var. *longifolia*. Total (2).

MYRTACEAE. Species (4): *Calyptranthes lilloi*, *C. oreophila*, *Eugenia guili*, *E. perorebi* Parodi ex Speg. & Girola. Infraspecific taxa (1): *Tepualia stipularis* var. *philippiana* (Griseb.) Speg. comb. nov. Total (5).

NYMPHAEACEAE. Species (1): *Cabomba australis*. Total (1).

ONAGRACEAE. Species (1): *Oenothera pygmaea*. Infraspecific taxa (3): *Oenothera odorata* var. *glabrescens*, *O. odorata* var. *media*, *O. odorata* var. *undulata*. Total (4).

ORCHIDACEAE. Species (11): *Chloraea albo-rosea* Kraenzl. ex Speg., *C. chica* Speg. ex Kraenzl., *C. cholilensis* Speg. & Kraenzl.*, *C. hookeriana* Speg. & Kraenzl.*, *C. hystrix* Speg. & Kraenzl., *C. phoenicea*, *C. pleistodactyla* Kraenzl. & Speg., *C. praecincta* Speg. & Kraenzl., *Epidendrum argentinense*, *Pleurothallis aurantio-lateritia*, *Restrepia cogniauxiana* Speg. & Kraenzl. Total (11).

OXALIDACEAE. Species (4): *Oxalis chubutensis* Speg. ex R. Knuth, *O. nahuelhuapiensis*, *O. patagonica*, *O. stenophylla*. Infraspecific taxa (1): *Oxalis valdiviensis* var. *humilis*. Total (5).

Table 2. Continued.

PHYTOLACCACEAE. Species (1): *Seguieria guaranitica*. Total (1).

PLANTAGINACEAE. Species (4): *Plantago carrenleofuensis, P. oxyphylla, P. pulvinata, P. tehuelcha*. Infraspecific taxa (10): *Plantago macrostachys* var. *subandina, P. maritima* var. *macrophylla, P. maritima* var. *pauciflora, P. myosuros* var. *hirta, P. myosuros* var. *latifolia, P. myosuros* var. *taraxacoides, P. patagonica* var. *gracilescens, P. patagonica* var. *minuscula, P. patagonica* var. *typica* nom. inval., *P. pauciflora* var. *taraxacoides*. Total (14).

PLUMBAGINACEAE. Species (1): *Statice patagonica*. Total (1).

POACEAE. Species (102): *Agrostis eremophila, A. kufuim, A. moyanoi, A. pyrogea, A. santacruzensis, A. tehuelcha, Andropogon agrostoides, Aristida pampeana, Atropis battandieri, Calamagrostis fuegiana, C. modesta, C. suka, Cortaderia dioica* (Spreng.) Speg. comb. nov., *Cryptochloris spatacea* nom. nud., *Deyeuxia ameghinoi, D. freticola, D. patagonica, Elymus chubutensis, E. leptostachyus, E. patagonicus, Festuca chubutensis, F. pampeana, F. pyrogea, F. shuka, F. ventanicola, Glyceria antarctica, G. fuegiana, G. leptostachys, Lappago oplismenoides, Leptocoryphium penicilligerum, Milium juncoides, Monanthochloe australis, Oplismenus oplismenoides* (Speg.) Speg. comb. nov., *Oryzopsis bicolor* (Vahl) Speg. comb. nov., *O. grisebachii, O. hackelii* (Arechav.) Speg. comb. nov., *O. lasiantha* (Griseb.) Speg. comb. nov., *O. lejocarpa, O. lejopoda, O. napostaensis, O. ovata, O. panicoides* (Lam.) Speg. comb. nov., *O. ruprechtiana* (E. Desv.) Speg. comb. nov., *O. stipoides* (Trin. & Rupr.) Speg. comb. nov., *O. tuberculata* (E. Desv.) Speg. comb. nov., *O. uruguayensis* (Griseb.) Speg. comb. nov., *O. verrucosa* (Phil.) Speg. comb. nov., *O. verruculosa, Panicum bambusoides* Speg. ex Arechav. hom. illeg., *P. guaraniticum, Poa argentina, P. chubutensis, P. erinacea, P. pungionifolia, P. yaganica, Savastana antarctica* (Labill.) Speg. comb. nov., *Spartina patagonica, Stipa ambigua, S. ameghinoi, S. arcaensis, S. arechavaletae, S. argentina, S. argentinensis, S. bavioensis, S. brachychaetoides, S. cacheutensis, S. caespitosa* (Griseb.) Speg. comb. nov., *S. calchaquia, S. chubutensis, S. cordobensis, S. curamalalensis, S. dasyantha, S. dasycnemis, S. gracilis, S. hypsophila, S. hystericina, S. jujuyensis, S. juncoides, S. leptothera, S. ligularis* (Griseb.) Speg. comb. nov., *S. molfinoi, S. nana, S. nubicola, S. oreophila, S. pampagrandensis, S. pampeana, S. paramilloensis, S. patagonica, S. perrigida, S. plagiostephana, S. psittacorum, S. psylantha, S. puelches, S. sanluisensis, S. scirpea* Speg. emend. Roig, *S. sublaevis, S. tehuelches, S. torquata, S. uruguaycola, S. uspallatensis, Triticum fuegianum, T. magellanicum* (Desv.) Speg. comb. nov. Infraspecific taxa (75): *Agrostis airoides* var. *flaccidifolia, A. moyanoi* var. *major, A. moyanoi* var. *plicatifolia, A. moyanoi* var. *puberigluma, Bromidium andinus* var. *scabrivalvus, Bromus andinus* var. *scabrivalvus, B. coloratus* var. *vivipara, B. unioloides* var. *hirsuta, B. unioloides* var. *humilis* hom. illeg., *B. unioloides* var. *micrantha, B. unioloides* var. *rupestris, Cortaderia quila* var. *patagonica, Danthonia picta* var. *patagonica, Deyeuxia hygrometrica* var. *tandilensis* (Kuntze) Speg. comb. nov., *Festuca erecta* var. *cirrosa, F. gracillima* var. *brevifolia, F. gracillima* var. *patagonica, F. myuros* var. *muralis* Kunth ex Speg., *Hierochloe redolens* var. *major, Hordeum murinum* var. *velutina, Oryzopsis bicolor* var. *major, O. bicolor* var. *media, O. bicolor* var. *minor, O. lejocarpa* var. *major, O. montevidensis* f. *brasiliensis, O. montevidensis* f. *trachycarpa, O. montevidensis* f. *typica, O. napostaensis* var. *brachyphylla, O. napostaensis* var. *brachysperma, O. napostaensis* var. *macrophylla, O. napostaensis* var. *typica, Poa bergii* var. *chubutensis, P. lanuginosa* var. *elata, P. scaberula* var. *gracillima, Polypogon elongatus* var. *patagonica, Stipa bavioensis* var. *minor, S. brachychaeta* var. *major, S. brachychaeta* var. *minor, S. caespitosa* var. *elata, S. caespitosa* var. *subelata, S. caespitosa* var. *subtypica, S. caespitosa* var. *typica, S. clarazii* var. *bulbosa, S. clarazii* var. *typica* nom. inval., *S. filiculmis* var. *major, S. filiculmis* var. *minor, S. humilis* var. *major, S. humilis* var. *minor, S. manicata* var. *latifolia* (Hack. & Arechav.) Speg. comb. nov, *S. manicata* var. *media, S. manicata* var. *typica* nom. inval., *S. megapotamia* var. *juncoides* (Speg.) Speg. comb. nov., *S. megapotamia* var. *typica* nom. inval., *S. papposa* var. *major, S. papposa* var. *minor, S. plumosa* var. *gracilis, S. plumosa* var. *media, S. plumosa* var. *micrura, S. setigera* var. *hispidula, S. setigera* f. *pallida, S. setigera* f. *purpurascens, S. setigera* f. *versicolor, S. speciosa* f. *major, S. speciosa* var. *minor, S. tenuis* var. *argentina* (Speg.) Speg. comb. nov., *S. tenuis* var. *typica, S. tenuissima* var. *oreophila* (Speg.) Speg. comb. nov., *S. tenuissima* var. *planicola, Triticum fuegianum* var. *patagonicum, T. magellanicum* var. *condensata* (J. Presl.) Speg. comb. nov., *T. magellanicum* var. *festucoides, T. magellanicum* var. *glabrivalva, T. magellanicum* var. *lasiopoda, T. magellanicum* var. *pubiflora* (Steud.) Speg. comb. nov., *T. magellanicum* var. *secunda* (J. Presl.) Speg. comb. nov. Total (177).

POLEMONIACEAE. Species (3): *Collomia chubutensis, C. patagonica, Gilia patagonica*. Infraspecific taxa (4): *Gillia gracilis* subvar. *spathulifolia, Navarretia involucrata* var. *pumila, N. involucrata* var. *trichophylla, Polemonium antarcticum* f. *violascens**. Total (7).

POLYGALACEAE. Species (6): *Acanthocladus moyanoi, A. tehuelchum, Polygala moyanoi* (Speg.) Speg. comb. nov., *P. oedipus, P. pamparum, P. tehuelchum* (Speg.) Speg. comb. nov. Total (6).

POLYGONACEAE. Species (3): *Coccoloba argentinensis, Eriogonum ameghinoi, E. bonaerense*. Infraspecific taxa (1): *Polygonum spectabile* var. *patagonica*. Total (4).

PORTULACACEAE. Species (8): *Calandrinia chubutensis, C. macrocarpa, C. patagonica, Portulaca amilis, P. cryptopetala, P. platensis, P. rosae, Talinum paraguayense*. Infraspecific taxa (3): *Portulaca cryptopetala* f. *phenopetala, P. oleracea* var. *macrantha**, *P. oleracea* var. *micrantha**. Total (11).

Table 2. Continued.

RANUNCULACEAE. Species (6): *Anemone myriophylla, Myosurus patagonicus, Ranunculus bovei, Ranunculus fuegianus, R. oligocarpus* hom. illeg., *R. potamogetonoides.* Infraspecific taxa (6): *Caltha sagittata* f. *latifolia, C. sagittata* f. *typica, Myosurus aristatus* var. *brachypoda, M. aristatus* var. *gracilis, Ranunculus bovei* var. *potamogetonoides* (Speg.) Speg. comb. nov., *R. peduncularis* var. *alboffiana.* Total (12).

RHAMNACEAE. Species (4): *Discaria andina**, *D. cognata* (Miers.) Speg. comb. illeg., *D. foliosa* (Hook. & Arn.) Speg. comb. illeg., *D. integrifolia.* Infraspecific taxa (4): *Colletia ferox* var. *puberula, Condalia lineata* var. *erythrocarpa, C. lineata* var. *melanocarpa, C. lineata* var. *xanthocarpa.* Total (8).

ROSACEAE. Species (10): *Acaena platyacantha, A. tehuelcha, Fragaria pampeana, Margyricarpus acanthocarpus, M. ameghinoi, M. niederleinii, M. patagonicus, Prunus argentinensis, Tetraglochin acanthocarpum* (Speg.) Speg. comb. nov., *T. ameghinoi* (Speg.) Speg. comb. nov. Infraspecific taxa (12): *Acaena multifida* var. *glaberrima, A. pinnatifida* var. *glabrata, A. platyacantha* f. *elata, A. platyacantha* var. *parvifolia, A. platyacantha* f. *typica, A. platyacantha* f. *villosa, Margyricarpus setosus* var. *patagonica, Tetraglochin acanthocarpum* var. *dasycarpa, T. acanthocarpum* var. *lasiocarpa, T. acanthocarpum* var. *leiocarpum, T. acanthocarpum* var. *macropoda, T. acanthocarpum* var. *typica* nom. inval. Total (22).

RUBIACEAE. Species (1): *Oreopolus patagonicus.* Infraspecific taxa (1): *Galium aparine* var. *pseudoaparine* (Griseb.) Speg. comb. nov. Total (2).

SAMYDACEAE. Genera (1): *Arechavaletaia.* Species (1): *Arechavaletaia uruguayensis.* Total (2).

SANTALACEAE. Species (2): *Acanthosyris platensis, Arjona ameghinoi.* Infraspecific taxa (4): *Arjona patagonica* var. *tandilensis* (Kuntze) Speg. comb. nov., *Quinchamalium chilense* var. *gracile* (Brongn.) Speg. comb. nov., *Q. chilense* var. *majus* (Brongn.) Speg. comb. nov., *Q. chilense* var. *patagonicum* (Berg.) Speg. comb. nov. Total (6).

SAPOTACEAE. Species (2): *Sideroxylon ligustrinum, S. myrtifolium* (Mart.) Speg. comb. nov. Total (2).

SAXIFRAGACEAE. Infraspecific taxa (3): *Saxifraga caespitosa* var. *pavonii, S. magellanica* var. *trilobata, S. trigyna* var. *patagonica.* Total (3).

SCROPHULARIACEAE. Infraspecific taxa (2): *Calceolaria bellidifolia* var. *angustifolia, C. lanceolata* var. *glabrata.* Total (2).

SIMAROUBACEAE. Species (1): *Picrasma palo-amargo.* Total (1).

SOLANACEAE. Genera (3): *Benthamiella, Pantacantha, Saccardophytum.* Species (27): *Benthamiella acutifolia, B. azorelloides, B. longifolia, B. patagonica, B. pycnophylloides, Fabiana patagonica, Grabowskia ameghinoi* (Speg.) Speg. comb. nov., *G. megalosperma, Himeranthus ameghinoi, H. patagonicus, Jaborosa desiderata, J. leptophylla, J. oxipetala, Lycium acanthocladium, L. ameghinoi, L. halophilum, L. lasiopetalum, L. repens, Nicotiana acaulis, N. ameghinoi, N. deserticola, N. sylvestris* Speg. & Comes, *Nierembergia patagonica, Pantacantha ameghinoi, Saccardophyton pycnophylloides, Solanum sidifolium, Trechonaetes leucotricha.* Infraspecific taxa (7): *Fabiana patagonica* var. *brachyloba, F. patagonica* var. *foliosa, F. patagonica* var. *gracilis, F. patagonica* var. *nana, F. patagonica* var. *typica* nom. inval., *Nicotiana alpina* var. *deserticola, N. alpina* var. *patagonica.* Total (37).

TROPAEOLACEAE. Species (1): *Tropaeolum patagonicum.* Infraspecific taxa (1): *Tropaeolum polyphyllum* var. *incisum.* Total (2).

VALERIANACEAE. Species (7): *Phyllactis carnosa, P. clarionifolia* (Phil.) Speg. comb. nov., *P. regularis, P. salicariaefolia* (Vahl) Speg. comb. nov., *Valeriana bonariensis, V. chubutensis, V. moyanoi.* Infraspecific taxa (3): *Phyllactis macrorhiza* var. *pumila, P. magellanica* var. *azorelloides, Plectritis samolifolia* var. *pusilla.* Total (10).

VERBENACEAE. Genera (1): *Monopyrena.* Species (15): *Lippia darwinii* (Benth. & Hook. f.) Speg. comb. nov., *Monopyrena serpyllifolia, Verbena ameghinoi, V. aurantiaca, V. azorelloides, V. carro, V. chubutensis, V. macrosperma, V. mulinoides, V. nubigena, V. patagonica, V. serpyllifolia* (Speg.) Speg. comb. nov., *V. silvestrii, V. struthionum, V. tandilensis.* Infraspecific taxa (3): *Verbena crithmifolia* var. *latiloba, V. flava* var. *angustiloba, V. flava* var. *latiloba.* Total (19).

VIOLACEAE. Species (1): *Viola argentinensis.* Infraspecific taxa (5): *Viola maculata* f. *calliantha, V. maculata* var. *tenuifolia, V. microphylla* f. *fimbriata, V. microphylla* f. *macropoda, V. microphylla* f. *micropoda.* Total (6).

ZYGOPHYLLACEAE. Species (1): *Larrea ameghinoi.* Total (1).

LYCOPODIACEAE. Infraspecific taxa (1): *Lycopodium scariosum* var. *patagonicum.* Total (1).

OPHIOGLOSSACEAE. Infraspecific taxa (1): *Botrychium lunaria* var. *antarctica.* Total (1).

Total: 33 genera; 696 species; 386 infraspecific taxa. TOTAL TAXA: 1115.

tinian journals as *Anales de la Sociedad Científica Argentina, Comunicaciones del Museo Nacional de Buenos Aires, Physis,* and *Revista de la Facultad de Agronomía de la Universidad Nacional de La Plata.* There are two papers listing all of Spegazzini's publications: one ranges from 1878 to 1919 (Scala, 1919) and the other from 1919 to 1926 (Molfino, 1929). The subjects of Spegazzini's publications are diverse: algae, fungi, vascular plants, anthropology, zoology, and medicine. His work on vascular plants

deals with agriculture, floristics, morphology, paleobotany, phytochemistry, phytopathology, taxonomy, botanical travels, and xylology.

Although his taxonomic work on vascular plants covers numerous families, he had a special interest in Cactaceae (Senet, 1926; Kiesling, 1984), Fabaceae, Orchidaceae, and Poaceae. As mentioned by Hauman (1923), his publications of new taxa have been subject to criticism because many of them were based on taxa already described by other botanists. Table 2 shows a total of more than 1000 names at the rank of genus, species, variety, subvariety, and form listed as Spegazzini new names in *Index Kewensis* (Anonymous, 1997) and the Gray Card Index Database. Relevant literature (Zuloaga et al., 1994; Zuloaga & Morrone, 1996, 1999a, b) has been consulted for determining the current status of these names. As a consequence, some of them resulted in illegitimate or invalid names, or illegitimate, superfluous, or new combinations. A more detailed analysis of the vascular plant names published by Carlos Spegazzini will be obtained with the production of the type specimens catalogue (Katinas et al., in prep.).

Carlos Spegazzini made some local floristic studies, mainly within the Province of Buenos Aires, such as in La Plata city, and in the mountain ranges of Tandil and Ventana. Although he started the first floristic study of the flora of the whole Buenos Aires Province, he did not finish this work.

Most of his travel accounts were first written as reports for his sponsoring institution, and then usually published in contemporary journals. His accounts of the trips were thorough and amusing, with plenty of details ranging from his salary to the phytogeography of the area. When he died, many publications remained unfinished, e.g., a new issue of *Revista Argentina de Botánica*, iconographies of the family Commelinaceae in Argentina, a monograph of the genus *Prosopis* (Fabaceae), a work on the genus *Senecio* (Asteraceae), a flora of Patagonia, and a catalogue of the vascular plants of Argentina with their scientific and vernacular names, including the Indian names (Senet, 1926).

Carlos Spegazzini was a hard worker who published practically every year of his scientific life, with 1925 being his most productive (with 20 papers), just one year before his death.

The Legacy

Carlos Luis Spegazzini was an influential force in the botanical community of Argentina. As a professor he nurtured enthusiasm and dedication among his students, extending his influence beyond botanical instruction and encouragement. Inspired by his knowledge of agriculture, he helped to solve many serious pest problems in Argentina. Moreover, Spegazzini's fascination with Patagonia led him to the creation of one of the most interesting and complete collections of vascular plants of this part of the world. His herbarium of vascular plants constitutes a fundamental witness to the past vegetation of Argentina, a valuable source of information that contributes to portraying the present-day loss of biodiversity. His work has been pivotal in awakening botanical science in Argentina and a general consciousness of the significance of plant and fungal systematics. Those in the botanical community who knew Carlos Spegazzini emphasized his clear judgment, accessibility, and sense of duty. The Institute and Arboretum in La Plata, his lectures, his publications, and prolific herbaria form the backbone of the legacy of Carlos Spegazzini, an Italian botanist who loved Argentina as his own country.

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PHENETIC SIMILARITY PATTERNS OF DIOECIOUS SPECIES OF *POA* FROM ARGENTINA AND NEIGHBORING COUNTRIES[1]

Liliana Mónica Giussani[2]

ABSTRACT

Dioecious species of *Poa* L. are mainly American with a total of 41 taxa in South America, 11 in North America, 2 in New Zealand, and 1 species in the Peri-Antarctic Islands. South American species, considered within *Poa* sect. *Dioicopoa*, are found mostly in Argentina but occur elsewhere in Bolivia, Brazil, Chile, Paraguay, and Uruguay. The present treatment considers the phenetic variation of dioecious species of *Poa* in South America. Thirty-seven taxa (34 species and three varieties) were examined, using numerical techniques. Multivariate analyses revealed a phenetic pattern among species based on linear relationships. Relationships of independent species as well as species complexes were established. As a consequence, new synonymy within most of these complexes, and a new variety, *Poa rigidifolia* var. *ibari*, are proposed herein. Bivariate analyses showed other non-linear patterns of variation. These results, together with univariate analyses, were used to select diagnostic characters. Sexual dimorphism was the major source of species variation, and ranges for pistillate and staminate specimens were also calculated. An identification key for all taxa is included.

Key words: Poa, dioecious species, numerical taxonomy, morphological variability, synonymy, sexual dimorphism.

Poa L. is one of the largest genera within Poaceae, including over 200 species according to Hartley (1961), Nicora (1978), and Kellogg (1985). Additionally, Clayton and Renvoize (1986), Soreng (1990), Watson and Dallwitz (1992), and Anton and Connor (1995) mentioned 400 to 500 taxa within *Poa*.

Poa is distributed worldwide, particularly at high altitudes and latitudes in both hemispheres; the taxon is largely absent from low areas of tropical regions (Hartley, 1961). It is a recognizable, well-defined (Clayton & Renvoize, 1986; Nicora & Rúgolo de Agrasar, 1987; Soreng, 1990), and monophyletic genus (Hartley, 1961; Kellogg, 1990).

Interspecific variation in *Poa* is due principally to quantitative diagnostic characters (Kellogg, 1990). Discrete morphological variables are mostly unreliable, often varying widely with the environment, and even within a single plant (Kellogg, 1985; Rúa, 1996). Species are frequently grouped into complexes based on their morphological similarities. These species complexes further comprise different chromosome numbers (Åkerberg, 1942; Kellogg, 1985). Taxonomists who have studied the genus (Bor, 1952; Marsh, 1952; Torres, 1969, 1970; Vickery, 1970; Nicora, 1978; Moore, 1983; Kellogg, 1985; Tateoka, 1985; Edgar, 1986) agree on the difficulty in delimiting taxonomic boundaries within *Poa*. Clausen (1961) and Soreng (1990) both considered that this variability in chromosome number results from polyploidy, introgression as well as apomixis. Phenotypic plasticity as stimulated by environmental variability also obscures species delimitation within *Poa* (Vickery, 1970; Giussani & Collantes, 1997).

Breeding systems are highly diverse within *Poa*. Perfect flowers are typical, but dioecism, gynomonoecism, and gynodioecism are also found among American species (Anton & Connor, 1995). Apomixis on exclusively pistillate plants or facultative apomixis on perfect flowers are both well documented (Connor, 1979, 1981; Kellogg, 1987).

Evolution of dioecism within *Poa* could have derived from gynodioecious species, following species

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migration from North to South America (Anton & Connor, 1995). Dioecism is well represented in the Americas: 11 dioecious species are distributed in the Northern Hemisphere, while 41 range southward. These South American species were generally treated as *Poa* subg. *Dioicopoa* (E. Desv.) J. R. Edm., although Soreng (1998a, b) considered them within *Poa* sect. *Dioicopoa* E. Desv., to conserve *Poa* subg. *Poa* as monophyletic. In order to maintain a uniform treatment of the genus *Poa*, the taxonomic delimitation proposed by Soreng (1998a, b) and based on a phylogenetic approach will be followed.

South American dioecious species are mainly distributed in Argentina and Chile (Marticorena & Quezada, 1985; Zuloaga et al., 1994). Parodi (1936) and Rosengurtt et al. (1970) cited three endemic species for Uruguay: *P. arechavaletae* Parodi, *P. uruguayensis* Parodi, and *P. megalantha* (Parodi) Herter. Smith et al. (1981) and Longhi-Wagner and Boldrini (1988) mentioned one endemic species in southern Brazil: *P. reitzii* Swallen. Nicora (1995) described one new species from Paraguay, *P. pedersenii* Nicora; and Hitchcock (1927) and Renvoize (1998) cited one dioecious species in Bolivia, *P. buchtienii* Hack. Although it was originally intended herein to consider all South American species of *Poa* sect. *Dioicopoa*, some taxa were not included due to the lack of material or difficulties in the identification of doubtful taxa, especially with Chilean material, and endemic species of Uruguay and Brazil.

Partial taxonomic revisions of some South American taxa (Parodi, 1936; Torres, 1969, 1970; Nicora, 1978; Moore, 1983) used quantitative characters as diagnostic features, although there was much overlap in their character ranges among species. Differences in pistillate floret hairiness were also utilized for species classification, with staminate specimens, which are generally glabrous, discounted (Nicora, 1978).

The aim of this study is to analyze the pattern of morphological variation within *Poa* sect. *Dioicopoa* based on pistillate and staminate plants. Grouping from multivariate analyses was used to look for similarity among taxa and this, along with study of types, helped circumscribe species and establish synonymy when necessary. An identification key is presented as the result of numerical analyses. This key is based on groups of selected characters that are strongly correlated, while unique characters were only used when they presented conspicuous discontinuities.

Materials and Methods

Species and Specimens Analyzed

Morphological variation among 34 dioecious species and three varieties was analyzed. A list of species is presented herein as Appendix 1 at the end of this manuscript. Species included in this study derive from the following regional taxonomic treatments: Nicora (1978) and Moore (1983) for Patagonia; Torres (1969, 1970) for Entre Ríos and Buenos Aires, Argentina; Parodi (1932, 1937, 1940, 1950, 1961, 1962) and Zuloaga et al. (1994) for Argentina; Hitchcock (1927) and Renvoize (1998) for Bolivia; Smith et al. (1981) and Longhi-Wagner and Boldrini (1988) for Brazil; Marticorena and Quezada (1985) for Chile; and Parodi (1936) and Rosengurtt et al. (1970) for Uruguay.

Three hundred seventy-six exsiccatae out of approximately 800 specimens examined by the author were analyzed and regarded as operational taxonomic units (OTUs) for numerical analysis (Appendix 2). The number of specimens considered per species varies according to their representation in herbaria. Some endemic species are represented by only a few specimens, while other widespread species are represented by 15 or more. Types of all taxa were seen, and most of these were included in the numerical analysis. Only types of *Poa bonariensis* (Lam.) Kunth, *P. iridifolia* Hauman, *P. lanuginosa* Poir., *P. prichardii* Rendle, and *P. stuckertii* (Hack.) Parodi were not recorded. An attempt was made to cover the full range of morphological variation used by earlier authors to discriminate taxa, and to reflect distributional range for all species considered. Voucher specimens are deposited in the following herbaria: BAA, BAB, CORD, LP, LPB, and SI.

Morphological Variables

Forty-four morphological characters, including anatomical and epidermal leaf characters, were measured for this numerical analysis. Of these, 29 are quantitative multistate characters and 15 are discrete variables (see Appendix 3). Most of them were considered diagnostic in previous taxonomic studies (Torres, 1969, 1970; Nicora, 1978; Moore, 1983). Others were added to increase the character database. Vegetative characters were measured on the penultimate leaf of a sterile innovation. Leaf anatomical characters were sampled on a cross section from the blade midregion. Epidermal characters represent the average of 10 measurements from the abaxial epidermis of the leaf blade midregion. Epidermal and anatomical characters follow the

character descriptions from Ellis (1976, 1979). Reproductive characters were measured on the tallest fertile culm.

ASSESSMENT OF THE TAXONOMIC COMPLEXES AND SPECIES

Phenetic similarities were examined by principal component analysis (PCA). This ordination technique represents distances between major groups more accurately than any clustering method (Sneath & Sokal, 1973). A correlation coefficient is suggested for mixed data with predominantly quantitative multistate characters (Crisci & López Armengol, 1983). Numerical analysis was performed on a standardized character matrix. Data were transformed by the Pearson-moment correlation coefficient on a similarity matrix, which was used to obtain the principal components. The basic data matrix is deposited in the Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina (Giussani, 1997: appendix B), and is also available from the author upon request.

PCA analysis was repeated several times in order to detect different morphological patterns within the species studied. Toward this, different steps of multivariate analysis were followed:

(1) A single species or similar species groups were recognized by means of PCA.

(2) Each species or species group was characterized by the contribution of each character to the first five principal components.

(3) Distinctive species or species groups were separated and re-analyzed to recognize another variation pattern.

(4) Species that were not initially delimited were re-analyzed until all taxonomic entities assorted into species complexes or were recognized as independent species.

Invariant characters were removed before PCA was performed. PCA distortion was measured due to the relatively low variability seen in the first five principal components. For this purpose, a Euclidean distance matrix deriving from the first five PCA axes was compared with a similarity matrix by a cophenetic correlation coefficient (Sneath & Sokal, 1973). Two dissimilarity matrixes were obtained based on two indexes: taxonomic distance coefficient and Manhattan distance, with r_T, and r_M the cophenetic correlation values, respectively. Of these, only the highest scored value is presented.

Minimum spanning tree or MST procedure (Gower & Ross, 1969; Rohlf, 1992) was additionally used to examine the similarity relationships among OTUs. This distance tree was imposed on the PCA plot with the OTUs linked by lines representing their minimum total distance (Clifford & Stephenson, 1975). MTS was performed on dissimilarity matrixes derived from taxonomic distance coefficient or Manhattan distance.

DISCRIMINATION AMONG TAXONOMIC ENTITIES

Taxonomic groups were first defined by PCA and then considered as a priori groupings for discriminant analysis or DA (Sneath & Sokal, 1973; Affifi & Clark, 1984). DA was performed to identify levels of certainty within previously recognized groups as well as to select diagnostic characters based on standardized coefficients of the canonical variables.

For discriminant analysis, only those characters that contributed most to the variability of the first five components of the PCA ($r > 0.5$) and that had the least correlation among each other ($r < 0.5$) were included. The only discrete characters analyzed by DA were those of diagnostic value for previous classifications (Torres, 1969, 1970; Nicora, 1978; Moore, 1983), but quantitative multistate characters were preferred.

The empirical method (Affifi & Clark, 1984) was used to estimate goodness of fit of the classification procedure. Thus, the discriminant function was applied to the same samples used for deriving it, and the proportion of individuals correctly classified for each group was computed.

UNIVARIATE AND BIVARIATE ANALYSES. A KEY FOR IDENTIFICATION OF SPECIES AND TAXONOMIC COMPLEXES

Mean and standard deviations for quantitative characters, as well as mode for discrete variables, were calculated for each species and species complex: these were plotted to reveal discontinuities and the pattern of variation among taxonomic entities (Sokal & Rohlf, 1969). Variation of a single character was also analyzed to consider dimorphism between pistillate and staminate plants. Thus, averages of pistillate and staminate plants were calculated. Bivariate analysis was used to detect morphological patterns based on different character combinations. After numerical analyses were completed, diagnostic characters were selected, and a key to identify species or taxonomic complexes was manually constructed.

Numerical analyses were made using either NTSYS-pc (Rohlf, 1992) or STATGRAPHICS (Statistical Graphics System by Statistical Graphics Corporation, 1992).

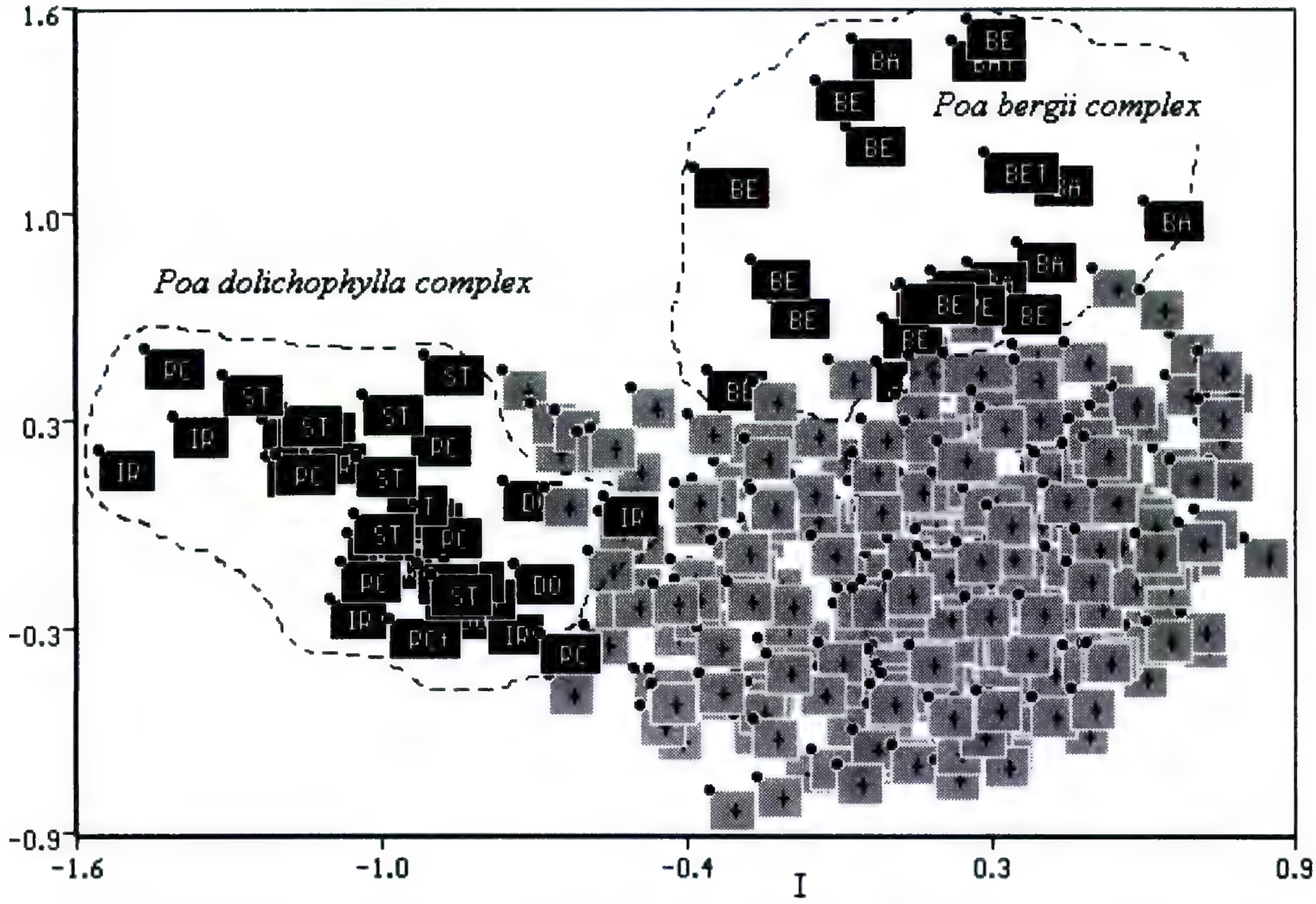


Figure 1. First set of multivariate analyses. Plot of 376 individuals on the first two principal components. Two groups of species are distinguished: the *Poa dolichophylla* complex (DO, IR, PC, ST), and the *P. bergii* complex (BE, BA). Each label corresponds to the species name, with the first two letters abbreviated as in Appendix 1 (pistillate individuals in all capital letters, and staminate individuals in lowercase letters); the third T or t letter indicates the type species; + = a non-grouped specimen.

Results

Taxonomic groups were selected based on the distribution of OTUs in the first five axes of PCA, since distortion of these axes was less than 10%.

First set of multivariate analyses

The *Poa dolichophylla* Complex, the *Poa bergii* Complex, and *Poa schizantha*. Ordination of all 376 OTUs on the first five axes, utilizing 44 characters, would account for 52.4% of total variability. Although this is a small percentage of total variability, PCA distortion was low: $r_M = 0.92$.

The first three principal components showed two groups of similar species. Axes I and II show the *Poa dolichophylla* complex, containing *P. dolichophylla* Hack., *P. pilcomayensis* var. *calamagrostoidea* Hack., *P. stuckertii*, and *P. iridifolia* (Fig. 1). These principally grouped along the negative edge of axis I. From analysis of character loadings, they are characterized by long leaves, blades, and sheaths; plant stature over 50 cm tall; broad blades; long and broad panicles; numerous vascular bundles with sclerenchyma girders on both abaxial and adaxial epidermis; short and truncate ligules; groups of sclerenchyma cells extending on abaxial and adaxial epidermis at blade margin; small stomata; and short spikelets, glumes, florets, and lodicules.

The second species group was defined by axes I, II, and III and comprises three taxa: *P. bergii* Hieron. var. *bergii*, *P. barrosiana* Parodi, and *P. schizantha* Parodi. Specimens from these species clustered at the positive end of these axes (Fig. 1). They also present long leaves, blades, and sheaths, tall plants, and long panicles. However, these species are distinguished from the previous group by longer ligules, thicker blades, larger stomata, longer spikelets, glumes, and florets, additional nerving on the first glume, as well as numerous vascular bundles with sclerenchyma girders only on abaxial epidermis.

The second group was analyzed separately. PCA was performed on a subset of 33 OTUs based on 39 morphological variables. *Poa bergii* var. *bergii*, *P. barrosiana*, and *P. schizantha* share some attributes that were revealed as invariant characters before PCA was performed: long rhizomes (HAB 2, see Appendix 3), no viviparous florets (FLOv 0), glabrous lemmas between nerves (HAlbet 0), no vascular bundles with only adaxial sclerenchyma girders (SCLad 0), and numerous silico-suberose

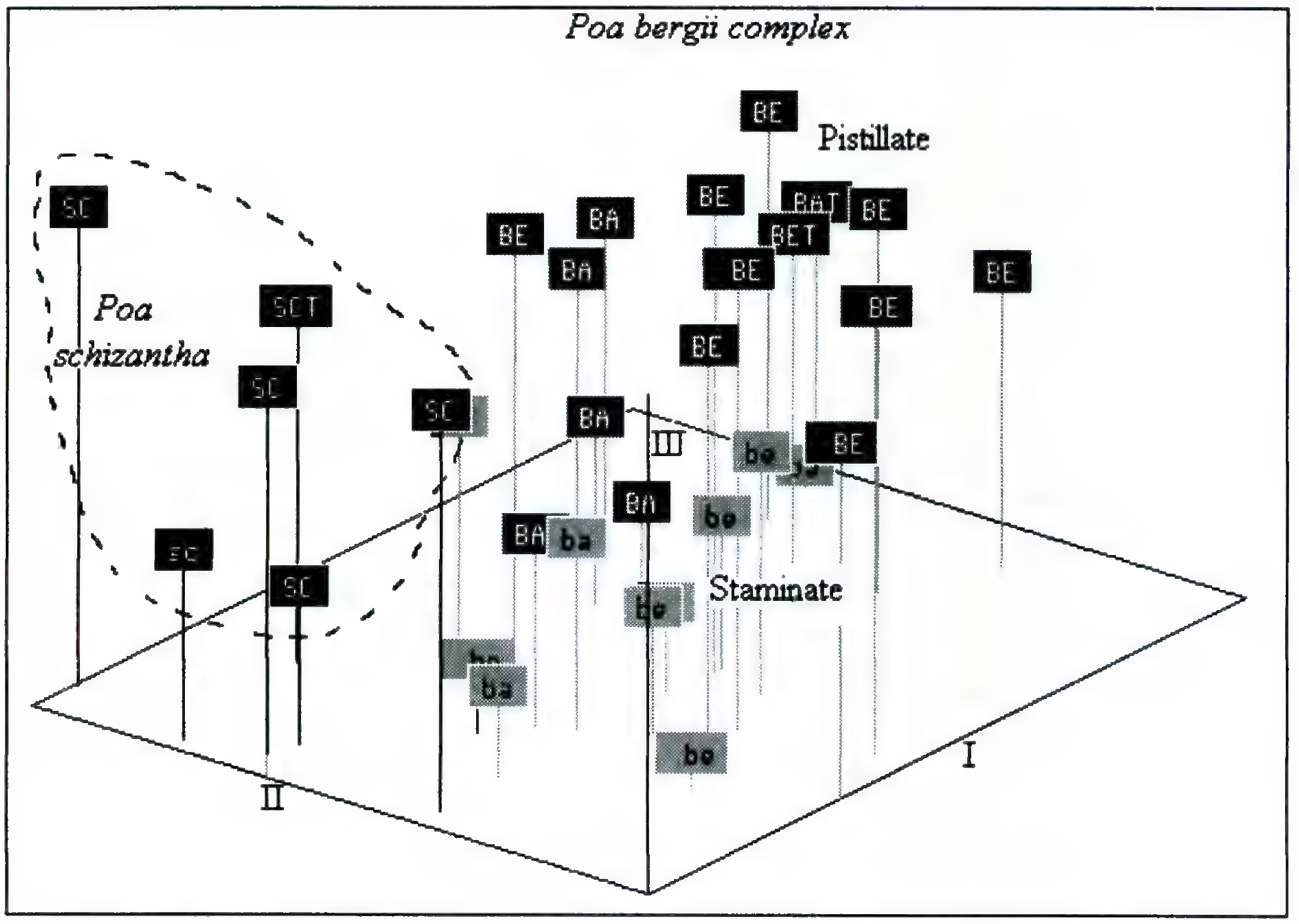


Figure 2. First set of multivariate analyses. Plot of 33 individuals on the first three PCA axes: *Poa schizantha* (SC) and pistillate and staminate individuals of the *P. bergii* complex are in different shades of gray. See Figure 1 caption for explanation of labels.

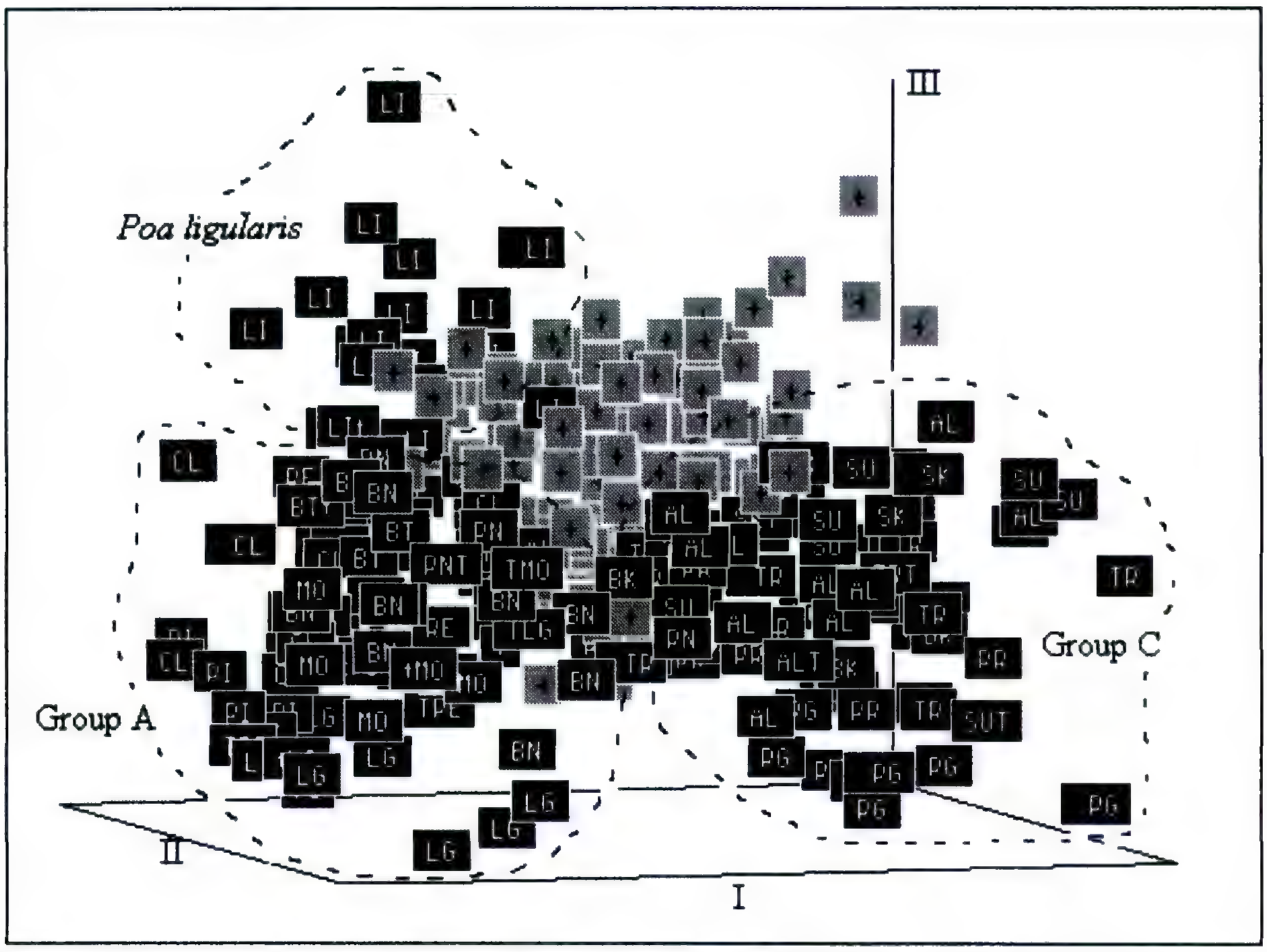
paired cells (SISU 2). These first five components accounted for 55% of total variation; meanwhile, distortion of PCA was low: $r_M = 0.93$. Specimens of *P. schizantha* were distinguished from those of the *Poa bergii* complex: *P. bergii* var. *bergii* and *P. barrosiana*; sexual dimorphism within the *P. bergii* complex was also supported. Figure 2 shows OTU distribution on the first three principal components. *Poa schizantha* falls on the negative end of axis I, and toward the positive ends of axes II and III. It is clearly defined by a smaller plant size; smaller, narrower, and thinner leaves; smaller fertile structures; long and rigid callus hairs; and convolute blades. It also has smaller stomata and bulliform cells only slightly or not differentiated from other epidermal cells. A clear dimorphism between pistillate and staminate plants of the *P. bergii* complex was also distinguished. Pistillate specimens associate with the positive end of axes I and III: they are larger plants, with more hairy florets than staminate specimens.

Discrimination among taxonomic entities. The *Poa dolichophylla* complex, the *P. bergii* complex (*P. bergii* and *P. barrosiana*), *P. schizantha,* and a remaining group of miscellaneous species comprised the four a priori groups for an initial discriminant analysis. Standardized coefficient values for the first three canonical variables were used to characterize groups. Species within the *P. dolichophylla* complex have wide leaves and blade margins with a group of sclerenchyma cells extending on the adaxial and abaxial epidermis; the *P. bergii* complex has long ligules, sheaths, lemmas, stomata, as well as numerous glume nerves. *Poa schizantha* is characterized by intermediate ligule, lemma, and stomata lengths, as well as narrower blades and thinner long cells. These groups appeared appropriately classified by discriminant functions. Eighty-eight percent of specimens of the *P. dolichophylla* complex, 85% of the *P. bergii* complex, and 100% of *P. schizantha* correctly assorted to the a priori groups.

SECOND SET OF MULTIVARIATE ANALYSES

After subtracting specimens of the *Poa dolichophylla* complex, the *P. bergii* complex, and *P. schizantha* from the original data matrix, the 302 remaining specimens were re-analyzed with the original 44 morphological and anatomical characters. The first five PCA axes accounted for 49% of total variability; in spite of this percentage, distortion was low at $r_M = 0.89$.

OTU distribution on the first three axes of PCA discriminates three groups of species (Fig. 3): GROUP A, constituted by *P. bonariensis, P. buchtienii, P. calchaquiensis* Hack., *P. lanigera* Nees, *P.*



Figures 3. Second set of multivariate analyses. Plot of 302 individuals on the first three principal components showing Group A (*Poa bonariensis, P. buchtienii, P. calchaquiensis, P. lanigera, P. patagonica* var. *neuquina, P. pedersenii, P. pilcomayensis, P. montevidensis,* and *P. resinulosa*), Group B (*P. ligularis*), and Group C (*P. alopecurus, P. boelckei, P. pogonantha, P. prichardii, P. shuka, P. superbiens,* and *P. tristigmatica*). See Figure 1 caption for explanation of labels.

patagonica var. *neuquina* Nicora, *P. pedersenii, P. pilcomayensis* Hack., *P. montevidensis* Arechav., and *P. resinulosa* Nees ex Steud; GROUP B, consisting only of specimens of *P. ligularis* Nees ex Steud.; and GROUP C, which includes *P. alopecurus* (Gaudich.) Kunth, *P. boelckei* Nicora, *P. pogonantha* (Franch.) Parodi, *P. prichardii, P. shuka* (Speg.) Parodi, *P. superbiens* (Steud.) Hauman & Parodi, and *P. tristigmatica* E. Desv. ex Gay.

Analysis of character correlation with the first three PCA axes revealed that species of Group A, placed on the positive end of axis I and the negative end of axis III, are characterized by long blades; short, truncate ligules; and smaller spikelets, glumes, florets, and lodicules. Group B, on the positive end of axes I and III but the negative end of axis II, also has shorter spikelets and florets, but is distinguished by its longer ligules, filiform leaves, smaller stomata, as well as smaller plants and inflorescences. Species of GROUP C aligned on the negative end of axis I but the positive end of axis II. These species are distinguished by their larger spikelets, glumes, and florets; wider, thicker blades; additional vascular bundles with sclerenchyma girders on both abaxial and adaxial epidermis; and longer stomata.

Discrimination among taxonomic entities. Groups A, B, C, and all remaining species were the four a priori groups used for the discriminant analysis (DA). Specimens assorting to Group A and B separated along the first canonical variable. They are characterized by long blades, long hairs along lemma nerves, and sharp leaf blade apices. Specimens of Group C are distinguished by longer glumes. *Poa ligularis,* separated by the second canonical variable, is discriminated by a long ligule and numerous vascular bundles with sclerenchyma girders only on the abaxial epidermis. Group A is also characterized by wide blades associated with the second variable. All groups were assigned by the classification procedure with the percentages of correct classification being 86%, 90%, and 80% for groups A, B, and C, respectively.

The *Poa bonariensis* Complex, *Poa lanigera*, *Poa pilcomayensis* var. *pilcomayensis*, and the *Poa resinulosa* Complex. Species assorted within Group A include *Poa bonariensis, P. buchtienii, P. calchaquiensis, P. lanigera, P. montevidensis, P. patagonica* var. *neuquina, P. pedersenii, P. pilcomayensis* var. *pilcomayensis,* and *P. resinulosa.* Their phenetic relationships were investigated by

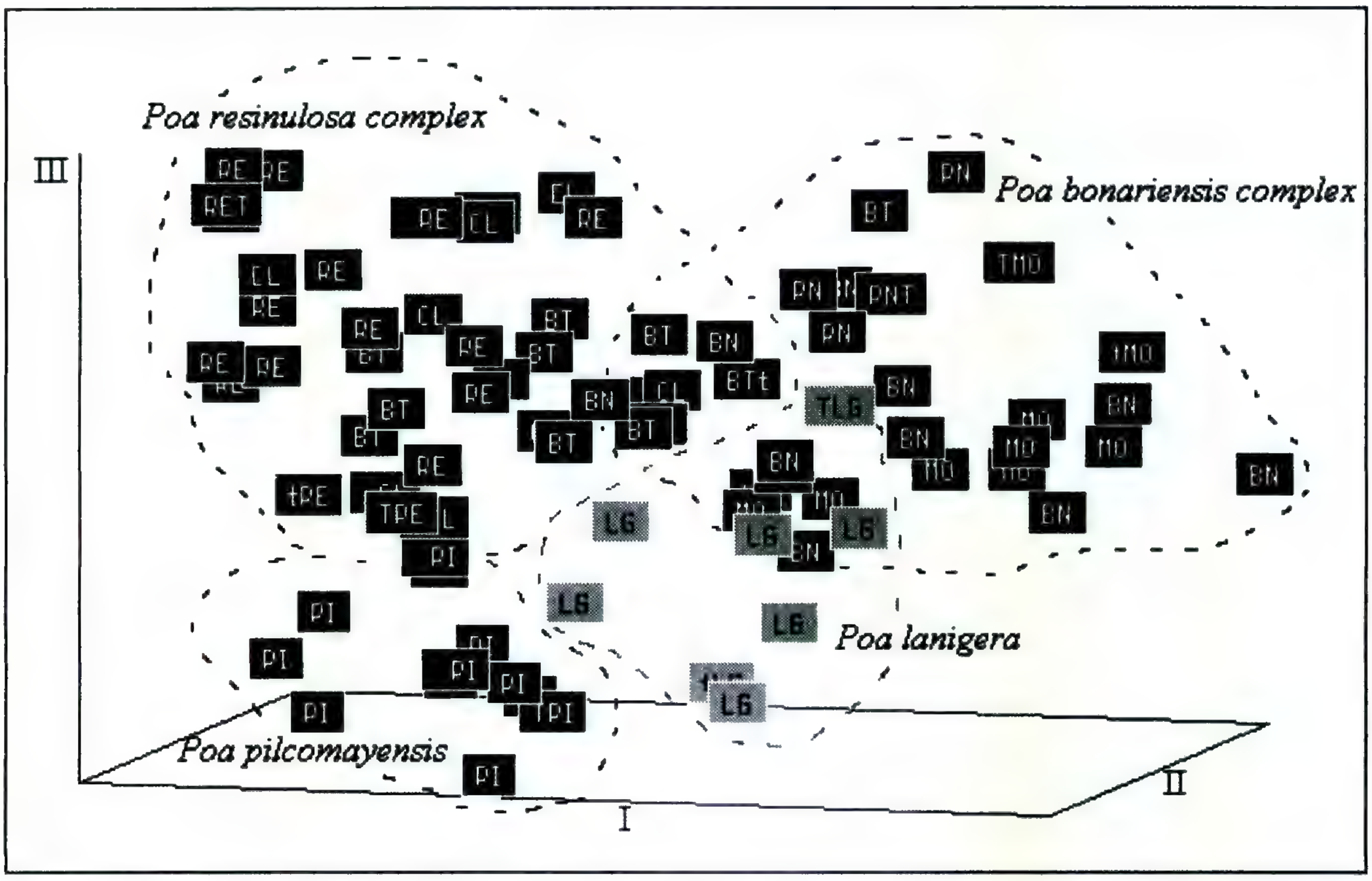


Figure 4. Second set of multivariate analyses. Plot of 84 individuals of Group A on the first three PCA axes showing the *Poa resinulosa* complex (BT, CL, PE, RE), the *P. bonariensis* complex (BN, MO, PN), *P. lanigera* (LG), and *P. pilcomayensis* (PI). See Figure 1 caption for explanation of labels.

PCA. A data matrix was based on 84 OTUs and 43 variables. Only 1 character out of the 44 was invariant: the absence of viviparous florets (FLOv 0, see Appendix 3). The first five components accounted for 53% of total variability. PCA distortion was low, being $r_M = 0.94$.

A plot of the first three principal components showed four distinct groups of species (Fig. 4). The *P. resinulosa* complex consists of *P. buchtienii, P. calchaquiensis, P. pedersenii,* and *P. resinulosa.* A *P. bonariensis* complex contains *P. bonariensis, P. patagonica* var. *neuquina,* and *P. montevidensis.* Two other groups each consist of two independent taxa, *P. pilcomayensis* var. *pilcomayensis* and *P. lanigera.* The *P. resinulosa* complex, located at the negative end of axis I and the positive end of axis III, comprises specimens of relatively small stature, with small stomata, narrow blades, as well as shorter spikelets, glumes, and florets. The *P. bonariensis* complex distributed to the positive end of axes I and III. This complex includes rhizomatous, larger plants, with longer stomata, wider blades, as well as larger spikelets, glumes, and florets. Vascular bundles with sclerenchyma girders are more numerous in blade cross section. *Poa pilcomayensis* var. *pilcomayensis* and *P. lanigera* both fell at the negative end of axis III. They share flat, narrow blades. However, *P. lanigera* is distinguished along the first principal component by its longer glumes, florets, and spikelets than are seen for *P. pilcomayensis* var. *pilcomayensis.*

Specimens within the *P. resinulosa* complex, including *P. buchtienii, P. calchaquiensis, P. pedersenii,* and *P. resinulosa,* were separately re-analyzed. PCA revealed a sexual dimorphism among specimens with staminate individuals presenting smaller fertile structures and less hairy florets than pistillate ones. The first five PCA axes accounted for 54% of the whole variance, and the cophenetic correlation coefficient showed a low PCA distortion, $r_M = 0.92$.

Specimens of the *P. bonariensis* complex were also separately analyzed. Principal differences among OTUs were again due to sexual dimorphism. This *P. bonariensis* group distributed on PCA axes I, II, and IV and was also sustained on a minimum spanning tree (Fig. 5). Staminate specimens, in contrast with pistillate specimens, again show smaller spikelets, glumes, and florets, as well as less hairiness on these florets.

Discrimination among taxonomic entities. A priori groups for DA corresponded to those obtained from PCA: (1) *P. bonariensis* complex; (2) *P. resinulosa* complex; (3) *P. pilcomayensis* var. *pilcomayensis*; and (4) *P. lanigera.* Two groups were separated along the first two canonical variables. The *P. resinulosa* complex is discriminated by a rela-

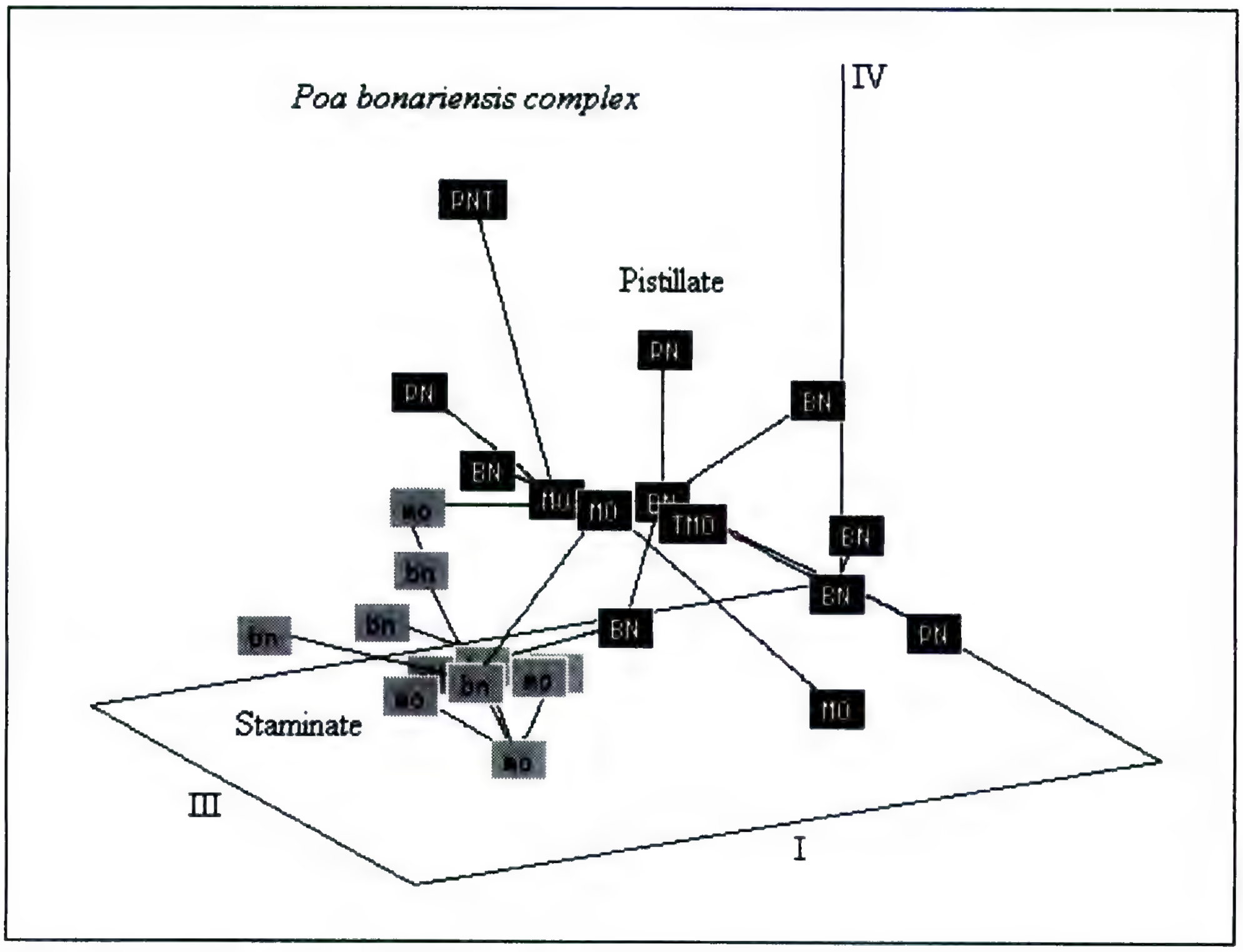


Figure 5. Second set of multivariate analyses. Plot of 25 individuals of the *Poa bonariensis* complex (BN, MO, PN) distributed on the I, III, and IV principal components. MST is superimposed on the PCA plot (lines). Dimorphism between pistillate and staminate specimens is the main source of variation among specimens of the complex. See Figure 1 caption for explanation of labels.

tively long ligule and subconvolute blades. The *P. bonariensis* complex is characterized by large panicles and glumes, wide paleas, numerous silico-suberose paired cells, and vascular bundles with sclerenchyma girders on the abaxial epidermis. *Poa pilcomayensis* var. *pilcomayensis* and *P. lanigera* aligned along the third canonical variable. *Poa lanigera* has broader paleas and hairier lemma nerves than *P. pilcomayensis*. Discriminant classification of the a priori groups showed a good fit: 92% of specimens for the *P. resinulosa* complex, 90% for the *P. bonariensis* complex, 92% for *P. pilcomayensis*, and 75% for *P. lanigera* were correctly assigned. Some misidentified specimens of the *P. resinulosa* complex associated with the *P. bonariensis* complex. Others of *P. pilcomayensis* were related to *P. lanigera*. Some specimens of *P. lanigera* intermixed with the *P. bonariensis* complex.

The *Poa alopecurus*, *Poa pogonantha*, and *Poa tristigmatica* Complexes. PCA ordination of specimens of Group C revealed two species groupings. The *Poa pogonantha* complex consists of *P. pogonantha* and *P. prichardii*, whereas specimens of *P. alopecurus*, *P. boelckei*, *P. shuka*, *P. superbiens*, and *P. tristigmatica* fall into the second group. OTU distribution (Fig. 6) is based on PCA axes I, II, and IV. Analysis of character loadings revealed that specimens within the *P. pogonantha* complex are characterized by viviparous florets and long panicles, but few have sclerenchyma girders associated with vascular bundles. A second species grouping features larger plants, longer ligules, as well as longer glumes, florets, and lodicules. The first five PCA axes explained only 47% of total variation. Nevertheless, its distortion remained low, $r_M = 0.87$.

Specimens of *P. alopecurus*, *P. boelckei*, *P. shuka*, *P. superbiens*, and *P. tristigmatica* were individually analyzed. Ordination along the first three principal component axes revealed two groups of species (Fig. 7): a *P. tristigmatica* complex with two species, *P. boelckei* and *P. tristigmatica*; and a *P. alopecurus* complex including *P. alopecurus*, *P. shuka*, and *P. superbiens*. The *P. tristigmatica* complex associated with the positive end of the first component axis due to smaller plants, leaves, and fertile structures. The *P. alopecurus* group principally located at the opposite side of axis I, associated with larger plants, leaves, and fertile structures. Pistillate and staminate specimens of *P. boelckei* and *P. tristig-*

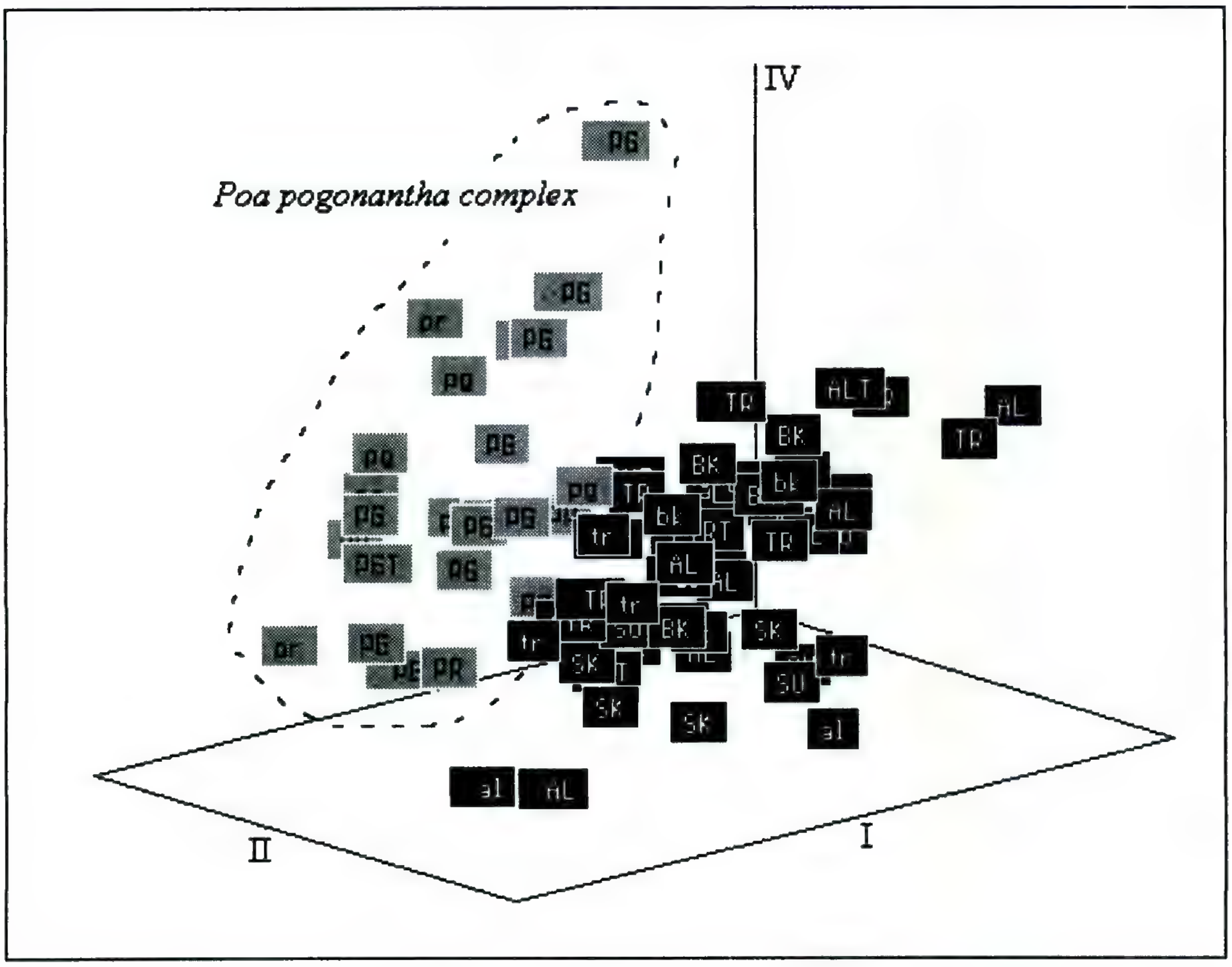


Figure 6. Second set of multivariate analyses. Plot of 76 individuals of Group C (*Poa alopecurus, P. boelckei, P. pogonantha, P. prichardii, P. shuka, P. superbiens,* and *P. tristigmatica*) on the I, II, and IV PCA axes. The *P. pogonantha* complex (PG, PR) is clearly distinguished from the remaining species. See Figure 1 caption for explanation of labels.

matica differentiated on axis II (Fig. 7). Pistillate plants present wider glumes and florets, longer lodicules, and thicker blades than staminate specimens. PCA distortion was low, at $r_M = 0.92$, although the first five axes accounted for just 49% of total variability.

When based only on specimens of the *P. pogonantha* complex, PCA revealed morphological differences between pistillate and staminate individuals. Pistillate specimens have hairy calluses, hairy lemma nerves, and vascular bundles with sclerenchyma girders on both abaxial and adaxial epidermis. Staminate specimens are distinguished by having more nerves on the first glume and well-differentiated bulliform cells. The first five axes accounted for 57% of total variability with PCA distortion being low, $r_T = 0.92$. Only three variables, navicular apices (API 1, see Appendix 3), no vascular bundles with few sclerenchyma cells (SCLin 0), and group of sclerenchyma cells at blade margin not extending on abaxial and adaxial epidermis (CAP 1) were invariant and removed from the matrix before performing PCA.

Discrimination among taxonomic entities. DA was performed to discriminate relevant groups emerging from the second ordination set. A priori groups were defined as: (1) the *P. tristigmatica* complex (*P. boelckei* and *P. tristigmatica*); (2) the *P. pogonantha* complex (*P. pogonantha* and *P. prichardii*); and (3) the *P. alopecurus* complex (*P. alopecurus, P. shuka,* and *P. superbiens*). Characters that best reflect specific differences were selected following standardized coefficients of canonical variables. The *P. tristigmatica* complex is characterized by broad glumes, paleas, and blades. The *P. pogonantha* complex is defined by long spikelets in association with viviparous florets, and long blades. Finally, the *P. alopecurus* complex has more nodes on the panicle along the principal axis, as well as more nerves on the first glume. These groups classified correctly: 91% were related to the *P. tristigmatica* complex; 82% to the *P. pogonantha* complex; and 96% to the *P. alopecurus* complex.

THIRD SET OF MULTIVARIATE ANALYSES

Specimens of species previously analyzed were removed from the data matrix. The 121 remaining OTUs were then considered for this subsequent study. PCA was performed; the first five components accounted for 48% of total variability. PCA distortion was low, $r_T = 0.91$. A group was differentiated along the I, II, and V axes, including *P.*

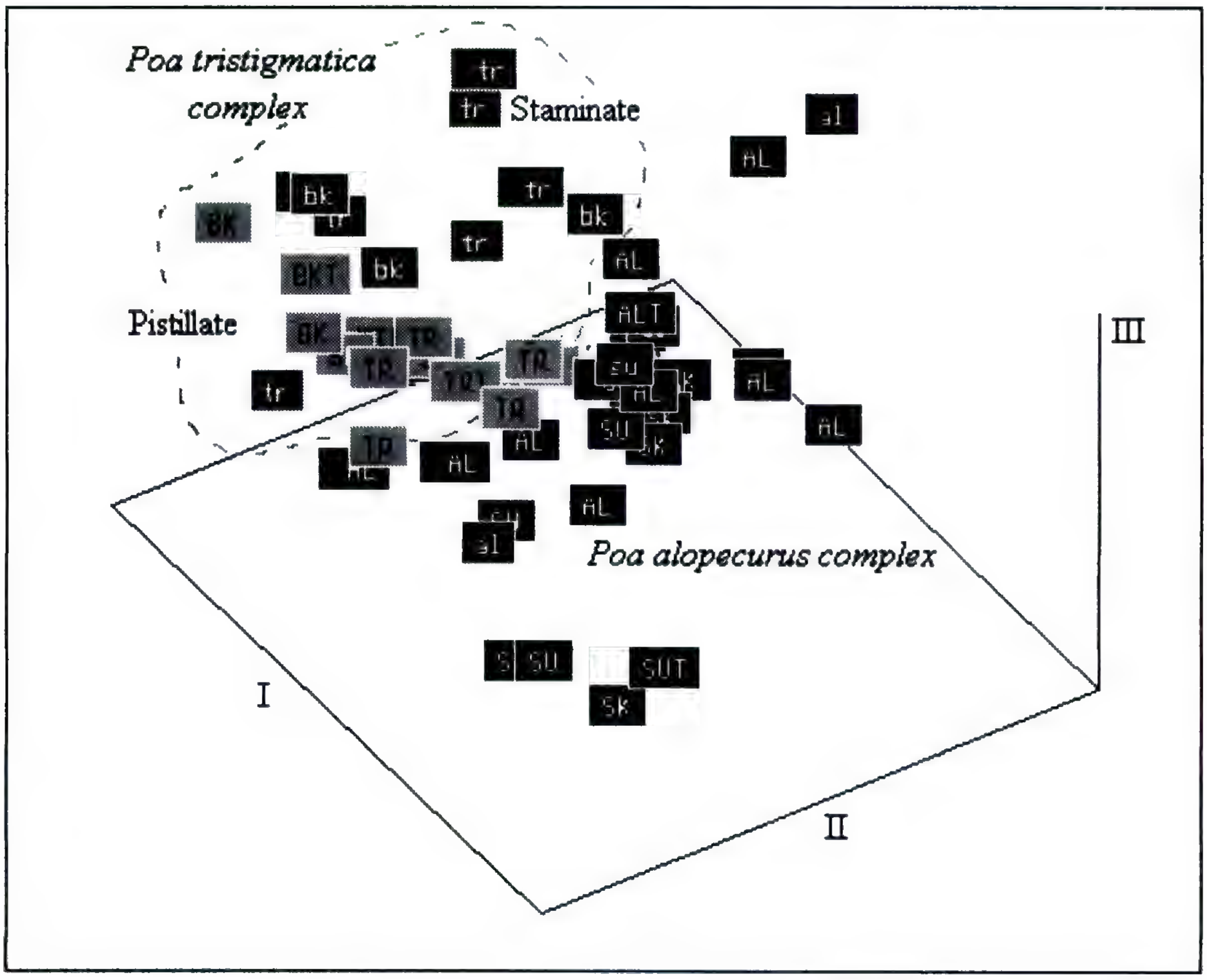


Figure 7. Second set of multivariate analyses. Plot of 52 individuals on the first three PCA axes showing two groups of species: the *Poa alopecurus* complex (AL, SK, SU) and the *P. tristigmatica* complex (BK, TR). See Figure 1 caption for explanation of labels.

bergii var. *chubutensis* Speg., *P. boecheri* Parodi, *P. hubbardiana* Parodi, *P. lanuginosa*, and *P. patagonica* Phil. var. *patagonica*. According to their character loadings, these species have longer leaves and inflorescences, wider and thicker blades, and larger stomata than the remaining species.

***Poa hubbardiana* and the *Poa lanuginosa* Complex.** The previously defined group (*Poa bergii* var. *chubutensis*, *P. boecheri*, *P. hubbardiana*, *P. lanuginosa*, and *P. patagonica* var. *patagonica*) was further analyzed by PCA. Figure 8 is based on the first three principal components. Two groups of species appear, with sexual dimorphism being the main source of variation within these groups. Caespitose *P. hubbardiana* separated along the third component and placed on its negative edge. This species is differentiated because it has a short ligule, small stomata, and long hairs lying between the nerves of the lemma. A *P. lanuginosa* complex (*P. bergii* var. *chubutensis*, *P. boecheri*, *P. lanuginosa*, and *P. patagonica* var. *patagonica*) principally distributed along the positive edge of axis III. Examining their character loadings, this group is defined by long ligules and small florets. Axes I and II showed a clear dimorphism between pistillate and staminate specimens of *P. hubbariana* as well as the *P. lanuginosa* complex. Pistillate plants are more robust, with longer spikelets, glumes, and florets, with these florets being distinctly hairy.

The first five components accounted for just 52% of total variability. However, the cophenetic correlation coefficient derived from PCA was high (r_M = 0.86), showing little distortion.

Discrimination among taxonomic entities. Species of the *P. lanuginosa* complex, *P. hubbardiana*, and the remaining specimens were used as a priori groups for DA. The first two canonical variables clearly discriminated these groups. The *P. lanuginosa* complex presents long panicles and large stomata. *Poa hubbardiana* has long florets, brief ligules, and sharp blade apices. Classification of the groups showed a few mismatches. Nevertheless, 91% of specimens of the *P. lanuginosa* complex were correctly classified. Meanwhile, 78% of expected specimens were associated with *P. hubbardiana*.

FOURTH SET OF MULTIVARIATE ANALYSES

***Poa holciformis*, *Poa huecu*, and *Poa indigesta*.** After discrimination and removal of *Poa hub-*

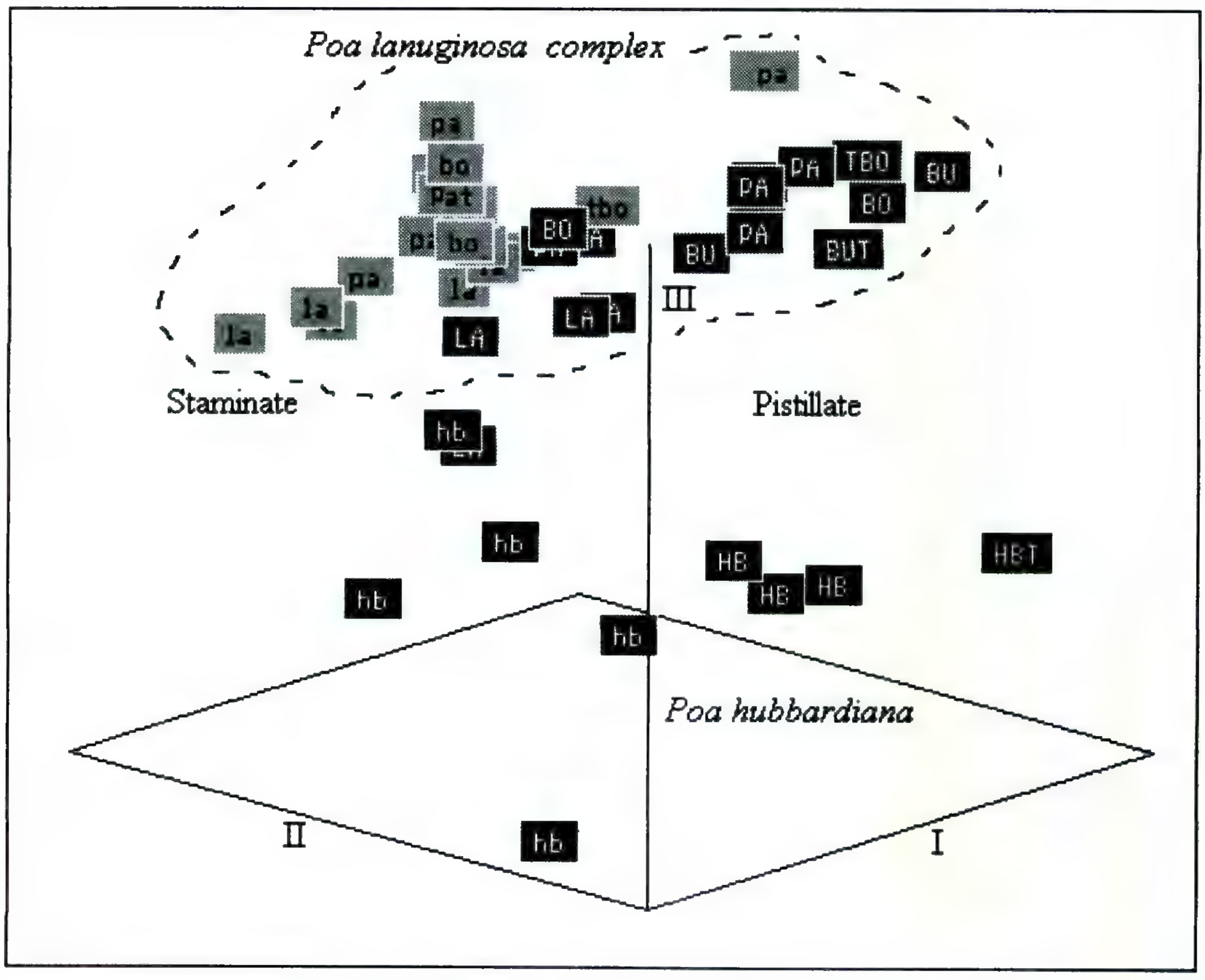


Figure 8. Third set of multivariate analyses. Plot of 43 individuals on the first three PCA axes: sexual dimorphism is clearly defined in *Poa hubbardiana* (HB) and the *P. lanuginosa* complex (BO, BU, LA, PA). See Figure 1 caption for explanation of labels.

bardiana and the *P. lanuginosa* complex, a new ordination analysis was performed on the 78 remaining specimens based on 43 morphological variables. One invariant character was excluded before performing PCA: groups of sclerenchyma cells at blade margins do not extend on abaxial and adaxial epidermis (CAP 1, see Appendix 3). The first five PCA axes explained 49% of total variability, with PCA distortion being low, $r_M = 0.91$.

OTU distribution on the first three axes showed a group of three caespitose species, *P. holciformis* Presl, *P. huecu* Parodi, and *P. indigesta* Parodi (Fig. 9). Specimens for those species principally associated with the positive end of axis I but the negative end of axis III. They are distinguished by their relatively long leaves and inflorescence, and relatively wide, thick blades. Spikelets, glumes, and florets are smaller than the same structures on the remaining species. Pistillate and staminate florets are glabrous.

Three species were independently analyzed: *P. holciformis*, *P. huecu*, and *P. indigesta*. These are recognized as single discrete entities on the first three PCA axes. *Poa indigesta* is the largest in size among the three taxa, and also has the longest, widest, and thickest leaves. *Poa holciformis* is differentiated from *P. huecu* by its longer spikelets, glumes, and florets, and wider blades. The first five components analyzed accounted for 59% of total variability, but their distortion was very low, with $r_M = 0.93$.

Discrimination among taxonomic entities. These previous three species were considered the a priori groups to discriminant analysis. Standardized coefficients of canonical variables revealed *P. indigesta* to present long blades, abundant cuticular prickles, and broad panicles. *Poa holciformis* is characterized by long lemmas and ligules. In contrast, *P. huecu*, at the opposite site, has shorter lemmas and more nerves on the first glume. Classification of specimens based on discriminant functions showed a good fit to data. One hundred percent of the specimens of a priori groups were included in *P. huecu* and *P. indigesta*, respectively. Eighty-three percent were correctly assigned to *P. holciformis*, but some specimens here associated with *P. huecu*.

FIFTH SET OF MULTIVARIATE ANALYSES

The *Poa denudata* and *Poa rigidifolia* Complexes. Upon the removal of *P. holciformis*, *P. huecu*, and *P. indigesta*, a new PCA involving the 54 remaining specimens and using 41 morpholog-

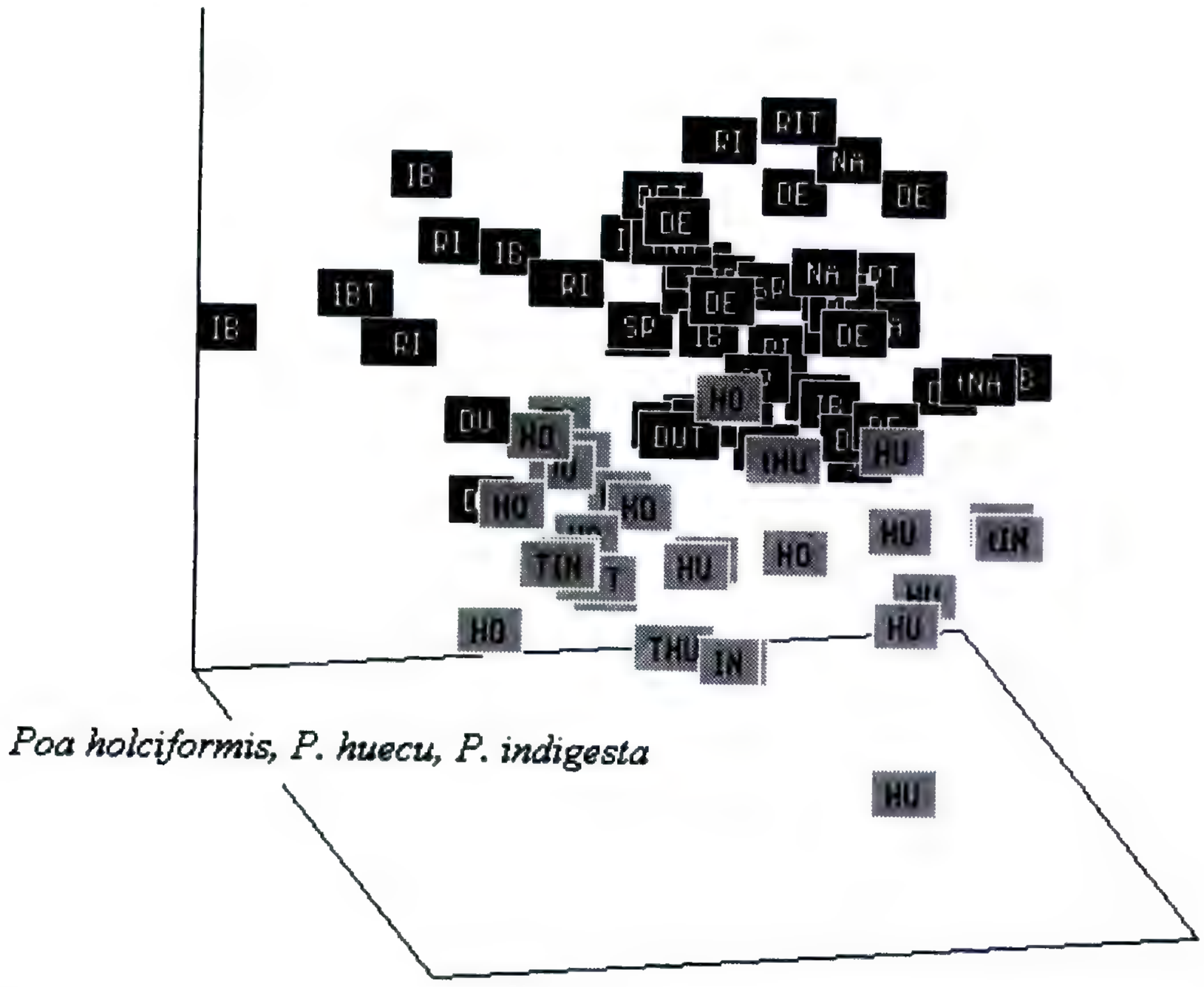


Figure 9. Fourth set of multivariate analyses. Plot of 78 individuals on the first three PCA axes showing *Poa holciformis*, *P. huecu*, and *P. indigesta*, clearly separated from the rest of species. See Figure 1 caption for explanation of labels.

ical variables was conducted. Three characters were excluded as invariant for the whole group. Apice blades are all navicular (API 1, see Appendix 3), viviparous florets are absent (FLOv 0), and all groups of sclerenchyma cells at blade margins do not extend on abaxial and adaxial epidermis (CAP 1). The first five principal components accounted for 50% of variance among specimens, yielding a low distortion for the analysis of $r_T = 0.91$.

Distribution of OTUs is represented on the first three principal components (Fig. 10). Specimens are gathered into two groups. The *Poa rigidifolia* complex (as defined by Giussani et al., 1996) consists of *P. dusenii* Hack., *P. ibari* Phil., and *P. rigidifolia* Steud. The *P. denudata* complex comprises *P. denudata* Steud. and *P. nahuelhuapiensis* Nicora. The *P. rigidifolia* complex principally associated with the negative end of axis I. This complex is distinguished by longer spikelets, glumes, and florets than seen in the *P. denudata* complex. Conversely, *P. denudata* and *P. nahuelhuapiensis* have longer leaves and inflorescences, wider blades, and more sclerenchyma girders on both sides of the vascular bundles. Minimum spanning tree or MST technique confirmed species groupings. This technique was performed because two specimens, the types of *P. dusenii* and *P. rigidifolia*, appeared isolated from their respective groups. Analysis of minimum distances among these OTUs showed relationship of the isolated types to the *P. rigidifolia* complex (Fig. 10).

Specimen ordination for only the *P. denudata* complex, *P. denudata* and *P. nahuelhuapiensis*, showed a clear dimorphism among pistillate and staminate individuals along the first three principal components. Pistillate specimens are characterized by hairy florets and longer leaves, inflorescences, and florets than staminate plants.

Discrimination among taxonomic entities. Specimens of both the *P. rigidifolia* and the *P. denudata* complexes were considered as a priori groups for DA and were characterized by analysis of standardized coefficients. The *P. denudata* complex presents long blades, large stomata, and broad panicles. Meanwhile, the *P. rigidifolia* complex presents long lemmas. Classification percentages showed a good fit between groups: 90% were included in the *P. denudata* complex, and 94% correctly classified into the *P. rigidifolia* complex.

UNIVARIATE AND BIVARIATE ANALYSIS

Previous analyses showed a phenetic pattern among dioecious species based on linear relationships, and it was possible to recognize complexes of similar species, as well as independent species.

Bivariate analysis revealed the correlation between different pairs of morphological and anatom-

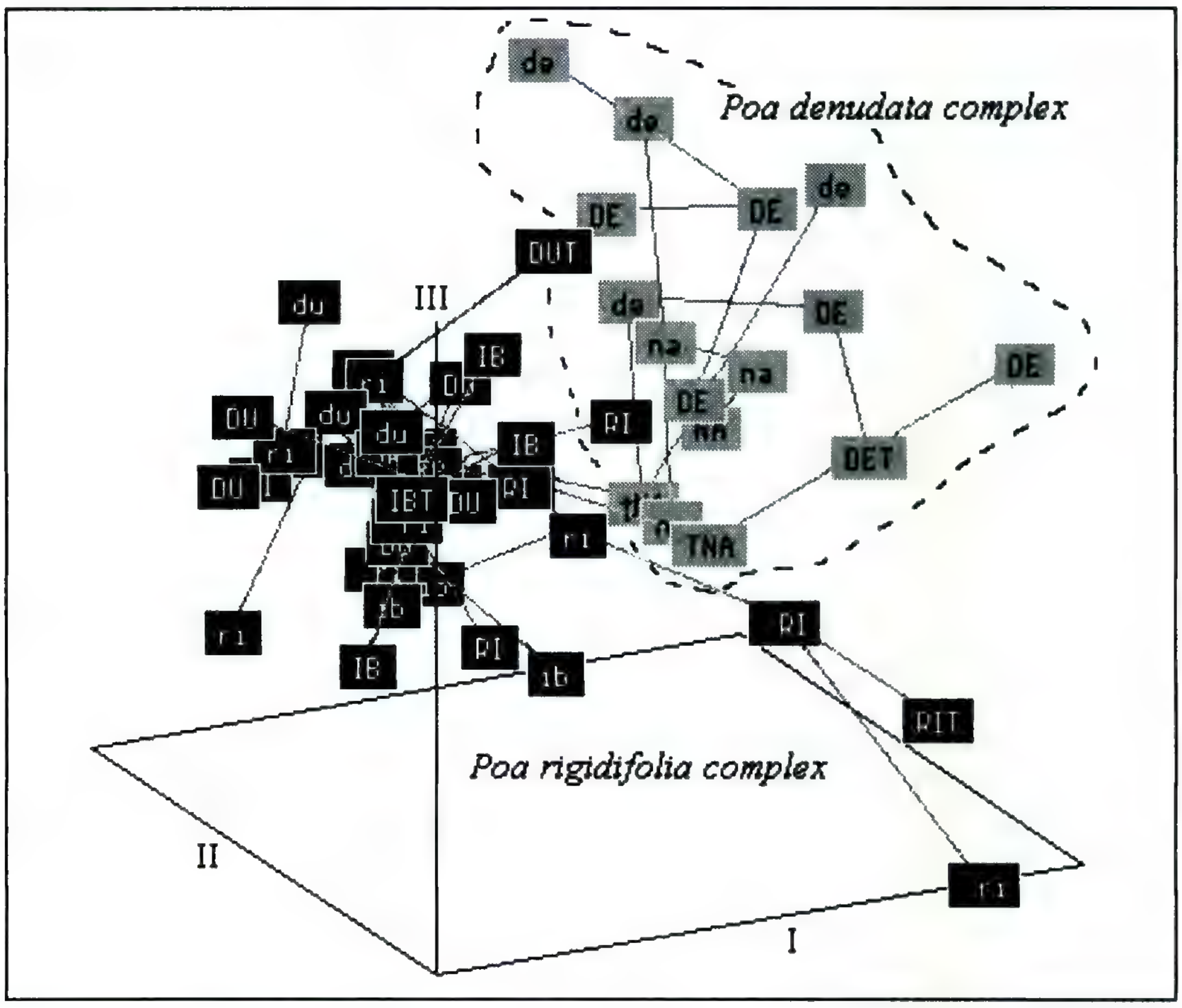


Figure 10. Fifth set of multivariate analyses. Plot of 54 individuals on the first three PCA axes and minimum total distances between individuals obtained from the minimum spanning tree represented by lines. The *Poa rigidifolia* complex is represented on black labels (DU, IB, RI), and the *P. denudata* complex is on gray labels (DE, NA). See Figure 1 caption for explanation of labels.

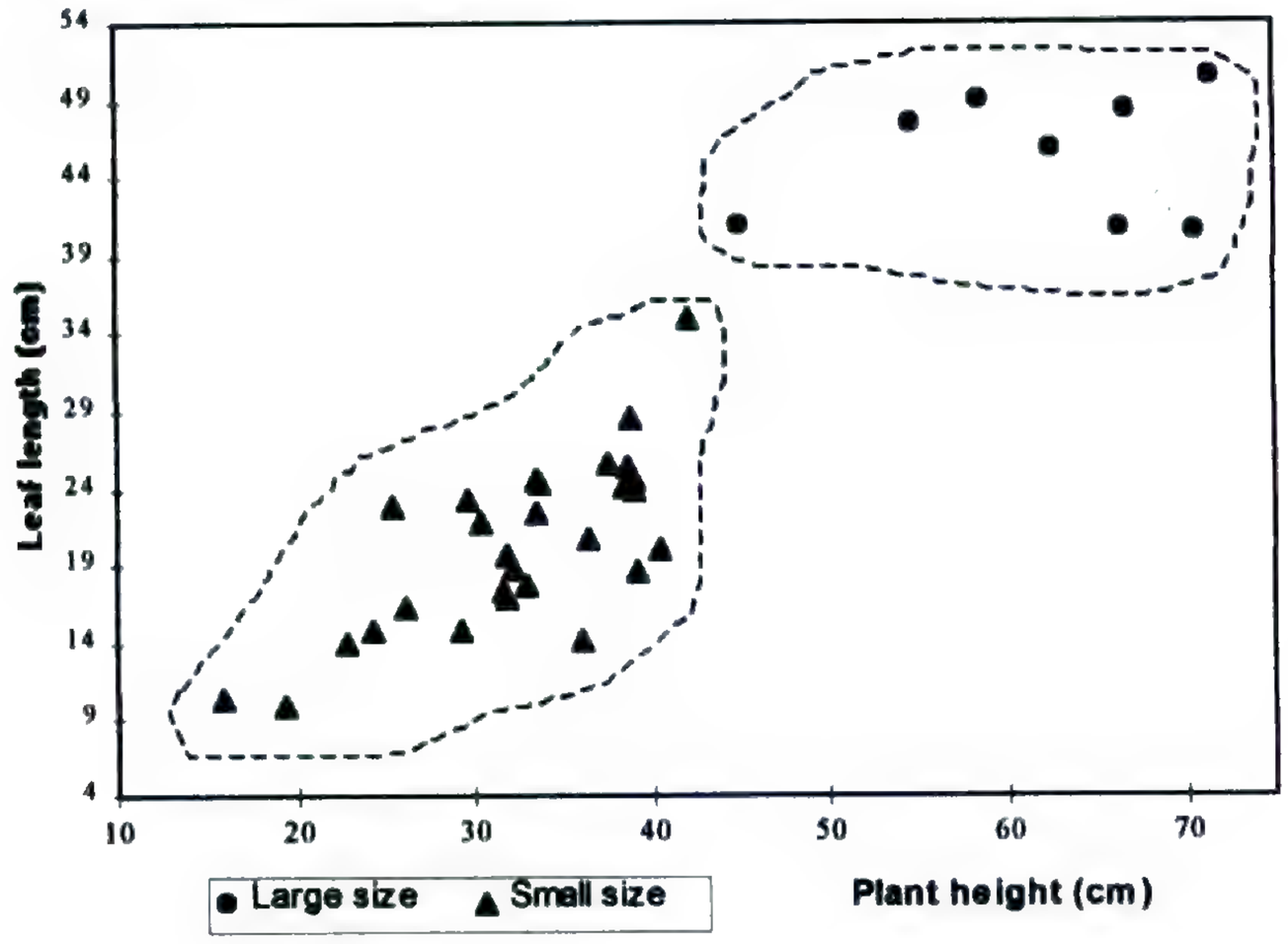
ical characters. Pairwise combinations were drawn for fertile characters (GLUle, GLUw, LEMle, LEMw, PALle, PALw, LODle, and LODw) as well as paired combinations of characters for relative plant sizes (LEAle, BLAle, SHEle, HEIG, PANle, PANn, PANw, BLAw, SCL2). All analyses showed linear relationships. Fertile characters are continuously distributed along the whole range of variation. In contrast, those characters for plant size present a conspicuous discontinuity between two main groups (Fig. 11). One of these main groups contains plants of large size including the *P. dolichophylla* complex (*P. stuckertii, P. iridifolia, P. pilcomayensis* var. *calamagrostoidea,* and *P. dolichophylla*), the *P. bergii* complex (*P. barrosiana* and *P. bergii* var. *bergii*), the *P. bonariensis* complex (*P. bonariensis, P. montevidensis,* and *P. patagonica* var. *neuquina*), and *P. indigesta.* Smaller plants distinguish the second group and include all remaining species.

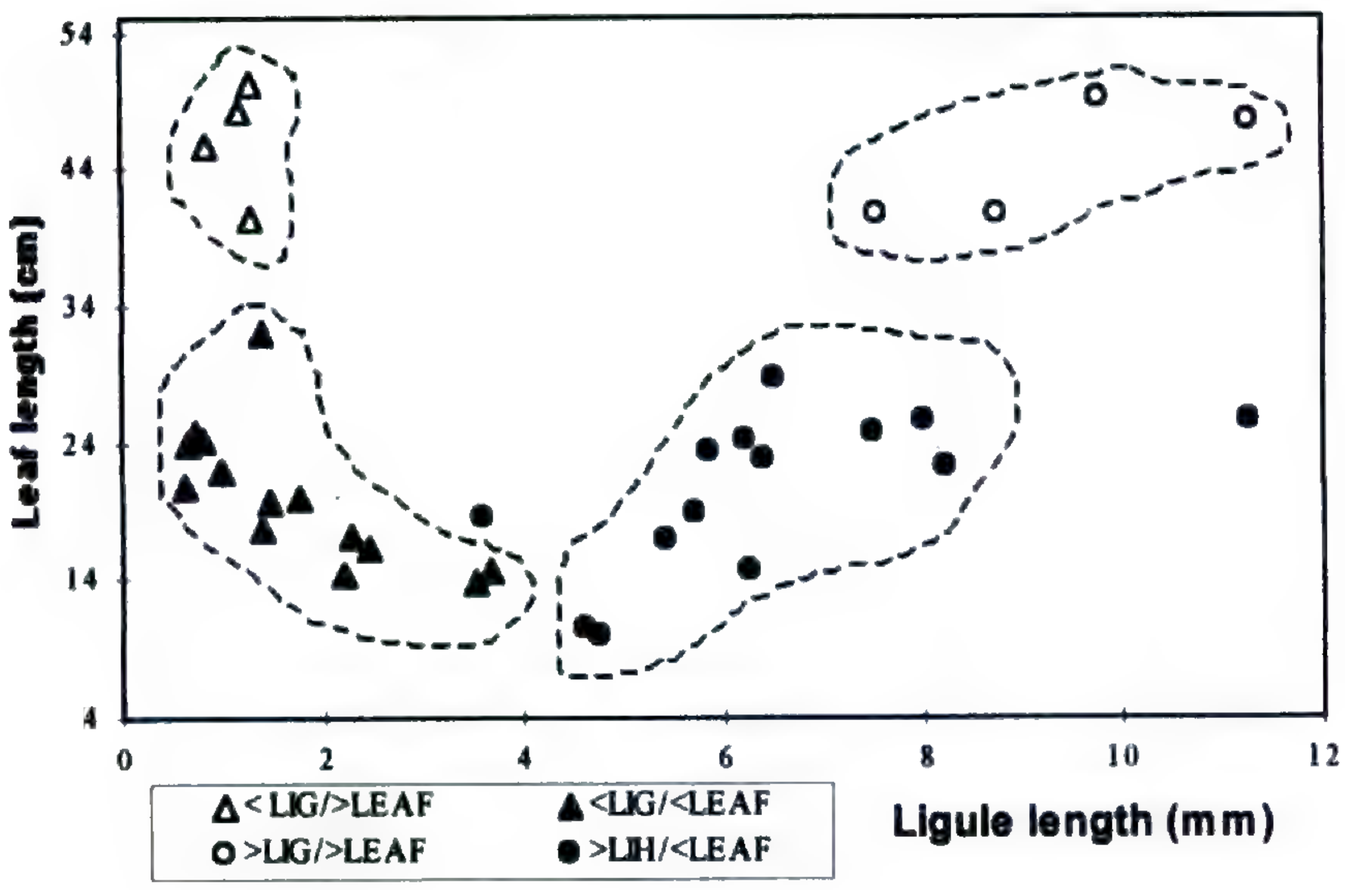
Another series of pairwise character combination showed a non-linear pattern of variation. Four taxonomic groupings are shown in Figure 12 and are based on the relation between ligule length and leaf length. One group includes species with short ligules but long leaves; a second species group, by contrast, has short ligules and short leaves. A third group combines species with both long ligules and long leaves. Finally, a fourth group consists of species with long ligules but short leaves. A similar grouping pattern was observed from the relationship between ligule length and any other character related to plant size (LEAle, BLAle, SHEle, HEIG, PANle, PANn). Another important character combination involves leaf length and stomata size (Fig. 13). Here, groups of species assembled according to: (1) small stomata but long leaves, (2) large stomata and long leaves, (3) large stomata but short leaves, and (4) short leaves but stomata of medium size.

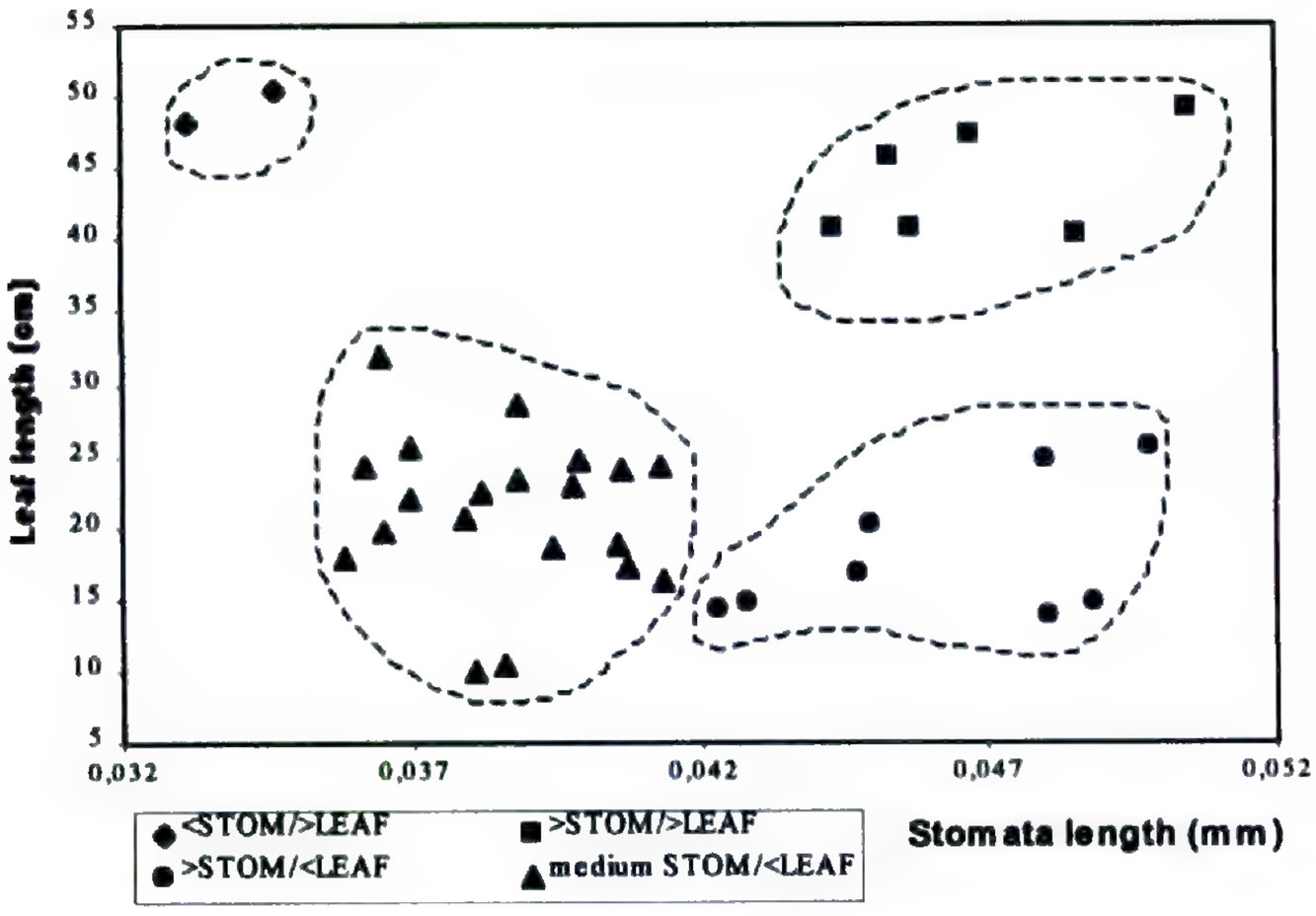
Univariate analysis considered the average (for quantitative variables) or mode (for discrete variables) for all complexes and individual species, as well as pistillate and staminate specimens, separately. Sexual dimorphism was noted on fertile characters related to spikelet, glume, and floret, as well as floret hairiness. Table 1 shows a list of selected characters representing the range of variation among complexes and species, as well as sexual dimorphism within entities.

Discussion

Taxonomic problems within *Poa* were accurately expressed by Bor (1952: 7–8), and his words re-







Figures 11–13. Bivariate analyses showing different patterns of morphological variation. —11 (top). Linear correlation pattern: plant height vs. leaf length. —12 (middle). Non-linear correlation pattern: ligule length vs. leaf length. —13 (bottom). Non-linear correlation pattern: stomata length vs. leaf length.

main relevant, as emphasized by more recent authors (Vickery, 1970; Soreng, 1990):

> "The systematic treatment of the species . . . is one of the most bewildering and difficult of taxonomic studies. While many species are clear-cut and can be recognized at a glance, there are groups of species about which one can only conclude that their evolutionary history has been so complex that they do not lend themselves to systematic treatment by present taxonomic methods. One cannot rely upon a single character to separate species in such groups, but combinations of more or less variable characters must be used. . . ."

This study of *Poa* sect. *Dioicopoa* utilized multivariate taxonomic techniques, as well as univariate and bivariate analyses, to better understand the morphological variation pattern—a different methodology from traditional taxonomic treatments. Analyses of both pistillate and staminate specimens denote complexes of species of great similarity, but it was also possible to clearly recognize some valid species. A multivariate key for identification among taxonomic entities of *Poa* sect. *Dioicopoa* was produced (see key herein).

Identification of varieties for three species analyzed here could not be supported. Thus, *Poa bergii* and *P. bergii* var. *chubutensis*, *P. patagonica* and *P. patagonica* var. *neuquina*, and *P. pilcomayensis* and *P. pilcomayensis* var. *calamagrostoidea* did not group together by similarity and were either included in different species complexes or maintained as independent entities. *Poa bergii* was grouped with *P. barrosiana* in a *P. bergii* complex, while its variety *P. bergii* var. *chubutensis* associated differently within the *P. lanuginosa* complex. *Poa patagonica* was more similar to species of the *P. lanuginosa* complex than to its variety *P. patagonica* var. *neuquina*, which clustered within the *P. bonariensis* complex. Finally, *P. pilcomayensis* was recognized as independent, while its variety *P. pilcomayensis* var. *calamagrostoidea* related to a *P. dolichophylla* complex.

Poa schizantha, a particular case within *Poa* sect. *Dioicopoa*, has been found only twice, and appears restricted to the dunes of Monte Hermoso, Argentina (Parodi, 1940). It is recognized by present results as a single species, related to the *P. bergii* complex. The three species (*P. barrosiana*, *P. bergii*, and *P. schizantha*) were found in similar environments and geographic areas. However, *P. schizantha* is clearly distinguished by a particular bilobed lemma, an interrupted panicle, and a different anatomical blade structure; among other characters, it lacks a well-developed midrib and bulliform cells. These features could originate by hybridization or odd mutation. Possibly, its offspring had low fertility and, whatever the case, it has never been collected again.

Plant stature of less than versus greater than 50 cm was a significant criterion by which to discriminate species within *Poa* sect. *Dioicopoa*. Plant height correlates with characters such as leaf blade and sheath length, panicle length, and number of nodes per panicle (Fig. 11). Other characters, of no linear variation, also address species discontinuities. Ligule length divides *Poa* sect. *Dioicopoa* into those species with short ligules (less than 2(–3) mm) and those with long ligules (more than (3–)4 mm).

Plant habit did not show, despite traditional treatments (Parodi, 1936; Torres, 1969, 1970; Nicora, 1978), a clear pattern of variation, nor any character correlation that could clarify the taxonomic structure within *Poa* sect. *Dioicopoa*. Some species could be differentiated only by the presence or absence of a rhizome. For example, species of the *P. resinulosa* complex, *P. pedersenii* and *P. resinulosa*, are caespitose, while *P. buchtienii* and *P. calchaquiensis* are rhizomatous. Parodi (1936) suggested that the rhizome is systematically valuable, dividing *Poa* sect. *Dioicopoa* by rhizomatous and caespitose habits. Later, Hunziker (1978) observed that crossings of rhizomatous and caespitose species of *Poa* produced fertile offspring, although the pattern of inheritance depended on the species that were crossed. Therefore, it remains preferable to group otherwise similar rhizomatous and caespitose species in the same taxonomic complex.

Sexual dimorphism was the principal source of variation within species and taxonomic complexes of *Poa* sect. *Dioicopoa*. Thus, it was necessary to establish ranges of variation for reproductive characters in pistillate and staminate specimens separately. Sizes of spikelets, glumes, and florets, as well as hairiness of florets, indicated major differences between pistillate and staminate plants. Traditionally, hairiness of the pistillate anthoecium was considered a useful discriminant character (Torres, 1970; Nicora, 1978). Since staminate florets are usually glabrous, it was not possible to investigate interspecific limits based on similar characters from staminate specimens. This study showed a great variation in hairiness of pistillate florets within, and among, analyzed groups. Therefore, these characters are less informative for interspecific variability. Callus hairiness on pistillate florets could be a synapomorphy for the whole genus or part of it (Kellogg, 1990), and is also valueless for interspecific variation. The absence of hairs on pistillate florets occurs only in *P. huecu*, *P. holciformis*, and *P. indigesta*. Both pistillate and staminate anthoecia in

Table 1. Morphological variation of species of *Poa* sect. *Dioicopoa* characterized by vegetative and reproductive characters. Median and standard deviation between parentheses for quantitative characters. Mode, and minimum and maximum values are between parentheses for variable discrete characters. Pi corresponds to number of pistillate specimens analyzed per species; St to number of staminate specimens. Character abbreviations as in Appendix 3.

Taxa	Leaf length (LEAle)	Ligule length (LIGle)	Plant height (HEIG)	Panicle length (PANle)	Nodes/ Panicle (PANn)	Blade width (BLAw)	Stomata length (STOM)
Poa alopecurus (Pi = 22, St = 8)	24 (± 9.86)	6 (± 2.2)	34 (± 16.11)	7.2 (± 2.28)	10 (6–15)	1.3 (± 0.35)	0.042 (± 0.0055)
P. bergii (Pi = 16, St = 11)	48.3 (± 14.92)	10.4 (± 3.48)	56.9 (± 14.46)	15.9 (± 5.1)	15 (11–20)	1.3 (± 0.44)	0.049 (± 0.0051)
P. bonariensis (Pi = 14, St = 11)	42.7 (± 13.42)	1.1 (± 0.61)	67.1 (± 20.59)	16.3 (± 4.13)	13 (10–18)	1.3 (± 0.29)	0.047 (± 0.0044)
P. denudata (Pi = 8, St = 8)	16.7 (± 7.06)	2.4 (± 1.61)	28.9 (± 6.29)	6.5 (± 1.81)	11 (9–14)	0.8 (± 0.18)	0.041 (± 0.055)
P. dolichophylla complex (Pi = 23, St = 18)	49.2 (± 18.57)	1.2 (± 0.66)	69.3 (± 19.56)	20.4 (± 5.85)	17 (11–20)	3.4 (± 0.98)	0.034 (± 0.0028)
P. holciformis (Pi = 8, St = 4)	16.2 (± 7.68)	5.7 (± 2.35)	29.2 (± 14.31)	7.6 (± 3.3)	10 (7–14)	1.3 (± 0.28)	0.044 (± 0.0044)
P. hubbardiana (Pi = 4, St = 5)	30.9 (± 11.62)	1.1 (± 0.41)	37.3 (± 13.51)	9.1 (± 2.57)	11 (8–13)	1 (± 0.21)	0.036 (± 0.0051)
P. huecu (Pi = 6, St = 3)	18.8 (± 8.32)	5 (± 2.2)	34.5 (± 11.94)	8.7 (± 1.02)	12 (10–16)	1.1 (± 0.17)	0.040 (± 0.0049)
P. indigesta (Pi = 2, St = 1)	40.8 (33.5–48.0)	7.9 (6.3–8.7)	57.6 (40–71.7)	18.5 (11.5–29)	15 (15–20)	1.5 (1.11–1.76)	0.048 (0.042–0.046)
P. lanigera (Pi = 5, St = 5)	24.3 (± 10.56)	0.7 (± 0.28)	36.2 (± 13.48)	9.2 (± 2.87)	13 (10–18)	1.5 (± 0.46)	0.040 (± 0.0039)
P. lanuginosa (Pi = 20, St = 14)	25 (± 10.21)	7.8 (± 3.19)	38 (± 15.44)	10.3 (± 4.11)	13 (9–18)	0.9 (± 0.22)	0.048 (± 0.0048)
P. ligularis (Pi = 12, St = 9)	22.6 (± 14.78)	7.4 (± 3.01)	29.8 (± 12.34)	7.3 (± 3.22)	12 (7–20)	0.7 (± 0.31)	0.038 (± 0.0037)
P. pilcomayensis (Pi = 5, St = 5)	21.3 (± 8.25)	0.8 (± 0.41)	33.3 (± 10.43)	9.3 (± 4.26)	8 (8–14)	0.9 (± 0.31)	0.037 (± 0.003)
P. pogonantha (Pi = 14, St = 9)	16.5 (± 6.58)	2 (± 1.08)	37.7 (± 16.37)	7 (± 2.32)	8 (6–12)	1 (± 0.30)	0.043 (± 0.0049)
P. resinulosa complex (Pi = 19, St = 20)	18.8 (± 12.39)	1.4 (± 1.01)	32.3 (± 17.71)	7.9 (± 4.23)	12 (8–16)	0.79 (± 0.22)	0.036 (± 0.0042)
P. rigidifolia (Pi = 23, St = 15)	9.7 (± 6.17)	4.7 (± 1.95)	17.2 (± 8.68)	4.3 (± 1.79)	9 (6–14)	0.7 (± 0.17)	0.037 (± 0.0056)
P. schizantha (Pi = 5, St = 1)	28.1 (± 6.45)	7.3 (± 2.31)	38.7 (± 6.94)	20.5 (± 6.57)	13 (9–13)	0.8 (± 0.26)	0.038 (± 0.0028)
P. tristigmatica (Pi = 14, St = 9)	14 (± 5.57)	3.6 (± 2.15)	26 (± 10.65)	6.5 (± 1.78)	10 (7–12)	1.6 (± 0.38)	0.048 (± 0.0048)

these three species are completely glabrous. This absence of hairs in these taxa could represent a loss with respect to the evolution of the entire genus, as suggested by Kellogg (1990) for other species of *Poa*. Pistillate anthoecia of *P. hubbardiana* are remarkably hairy, and clearly differentiated from other species. Its woolly hairs on the floret callus are numerous, long, and folded, while hairs on and between palea and lemma nerves are curled, and twice or three times longer than those seen in other species.

Out of the 34 species analyzed, only 8 were discriminated by numerical taxonomic methods: *Poa holciformis, P. hubbardiana, P. huecu, P. indigesta, P. lanigera, P. ligularis, P. pilcomayensis,* and *P. schizantha.* The remaining species showed congruent morphologies, aggregating into species complexes by phenetic similarities. Any hierarchical assignment to taxonomic varieties was not consistent with current species alignments. The validity of species assorting into complexes remains controversial. For classification purposes, a species comprises a taxonomic grouping of pistillate and staminate specimens of sympatric populations, sharing

Table 1. Extended.

Glume length (GLUle)		Lemma length (LEMle)		Hairs on callus (HAIcal)		Hairs on nerves (HAInrv)	
Pistillate	Staminate	Pistillate	Staminate	Pistillate	Staminate	Pistillate	Staminate
6.8 (± 1.16)	6 (± 1.27)	7.5 (± 0.98)	7 (± 1.42)	4 (0–4)	4 (0–4)	2 (0–3)	1 (0–3)
8 (± 1.25)	5 (±1.00)	8 (± 0.99)	6 (± 0.95)	4 (0–4)	0 (0–3)	3 (0–3)	0 (0–1)
4.3 (± 0.62)	3.2 (± 0.48)	5.3 (± 0.74)	4 (± 0.25)	4	4 (0–4)	3 (2–3)	0 (0–2)
4.3 (± 0.73)	3.6 (± 0.39)	5.3 (± 0.48)	4.5 (± 0.33)	4 (0–4)	0 (0–4)	2	0 (0–2)
3.4 (± 0.72)	2.9 (± 0.78)	4.1 (± 0.68)	3.6 (± 0.53)	4 (0–4)	0 (0–4)	2 (2–3)	0 (0–1)
4.7 (± 0.69)	3.7 (± 0.72)	5.4 (± 0.77)	4.7 (± 0.86)	0	0	0	0
5.8 (± 1.31)	4.2 (± 0.51)	6.6 (± 1.35)	5 (± 0.23)	4	4 (0–4)	3	0 (0–3)
3.4 (± 0.67)	2.8 (± 0.70)	4.1 (± 0.63)	4.1 (± 0.68)	0	0	0	0
3.7	2.5	4.5	3	0	0	0	0
4.2 (± 0.37)	3.2 (± 0.43)	5.3 (± 0.53)	3.8 (± 0.42)	4	0 (0–4)	3 (2–3)	2 (0–2)
5.1 (± 1.03)	3.8 (± 0.71)	5.8 (± 0.99)	4.7 (± 0.61)	4 (0–4)	4 (0–4)	3 (2–3)	0 (0–2)
3.6 (± 0.67)	2.7 (± 0.35)	4.8 (± 0.83)	3.4 (± 0.24)	4	0 (0–4)	3 (2–3)	0
2.9 (± 0.37)	2.5 (± 0.51)	4.1 (± 0.51)	3.4 (± 0.46)	4	4 (0–4)	2	0 (0–1)
6 (±0.79)	5.6 (± 1.28)	7 (± 1.12)	6 (± 0.59)	2 (0–4)	0 (0–3)	2 (1–2)	0 (0–2)
3 (± 0.71)	2.4 (± 0.42)	4 (± 0.79)	3.3 (± 0.43)	4 (0–4)	0 (0–4)	3 (0–3)	0 (0–2)
5.1 (± 0.95)	4.9 (± 0.82)	5.9 (± 0.79)	5.6 (± 0.85)	4 (0–4)	0 (0–3)	2 (2–3)	0 (0–2)
5.7 (±1.32)	4.1	6.6 (± 1.51)	5	2	0	0 (0–2)	0
5.5 (± 0.86)	4.5 (± 0.69)	6.7 (± 0.97)	5.4 (± 0.83)	2 (0–4)	0 (0–4)	2 (1–2)	0 (0–1)

similar variation pattern for several characters, not a single one. In general, morphological variation presents clear discontinuities identifying species. Moreover, the predominant variation pattern of a species is clearly associated with sexual dimorphism. Morphological variation within only pistillate plants or staminate specimens, correlated with environmental factors, appears significant only at the infraspecific level. By these criteria, sympatric species of *Poa* sect. *Dioicopoa* within the same taxonomic complex are synonymized herein. One new variety of *P. rigidifolia* is further proposed based on sexual dimorphism associated with environmental discontinuities. Precise ranges of morphological and anatomical variation are established herein and also correspond to Giussani (1997).

Taxonomic Treatment

Poa alopecurus (Gaudich. ex Mirb.) Kunth, Revis. Gramin. 1: 116. 1829. *Arundo alopecurus* Gaudich. ex Mirb., Ann. Sci. Nat., Bot. 5: 100: 1825. TYPE: Falkland Islands, East Falkland: Port Louis, 14 Feb.–28 Apr. 1820, *C. Gaudichaud s.n.* (holotype, P not seen, isotype, US 78849 [fragment ex P] not seen).

Poa shuka (Speg.) Parodi, Revista Argent. Agron. 20(4): 180. 1953. *Festuca shuka* Speg., Anales Mus. Nac. Hist. Nat. Buenos Aires 5: 95. 1896. TYPE: Argentina. Tierra del Fuego e Is. del Atlántico Sur: Ushuaia, Isla de los Estados, Port Vancouver, Blossom Bay, *C. Spegazzini s.n.* (holotype, LPS 14322 not seen; isotype, LP!).

Poa superbiens (Steud.) Hauman & Parodi, Physis (Buenos Aires) 9: 344. 1929. *Aira superbiens* Steud., Syn. Pl. Glumac. 1: 424. 1854. TYPE: Chile. Magallanes: Sandy Point, Dec., *W. Lechler 1194* (holotype, P not seen; isotype, BAA fragment!, US 2695872 ex Hb. Cosson P not seen, US 76311 ex W not seen).

This species inhabits the southern portion of Patagonia, covering western and southern regions of Santa Cruz, western areas of Tierra del Fuego e Islas del Atlántico Sur, Argentina, as well as boundary zones of XII Región, Chile.

Poa bergii Hieron., Bol. Acad. Nac. Ci. 3: 374. 1879. TYPE: Argentina. Río Negro: boca de Río Negro, *C. Berg 205* (holotype, CORD!).

Poa barrosiana Parodi, Physis (Buenos Aires) 11: 134. 1932. TYPE: Argentina. Buenos Aires: Miramar, 31 Jan. 1930, *L. R. Parodi 9820* (holotype, BAA!; isotype, US 89694 not seen).

Poa bergii inhabits costal dunes of Buenos Aires and Río Negro provinces, Argentina. It may extend to similar habitats in Uruguay and Brazil.

Poa bonariensis (Lam.) Kunth, Revis. Gramin. 1: 115. 1829. *Festuca bonariensis* Lam., Tabl. Encycl. 1: 192. 1791. TYPE: Argentina. E. Bonaria Circa Monte-Video, inter rupes et maritimas, 1767, *E. Commerson s.n.* (holotype, P not seen; US 2875384 ex P not seen; isotype, BAA fragment!).

Poa montevidensis Arechav., Anales Mus. Nac. Montevideo 1: 479. 1897. TYPE: Uruguay. Montevideo: en parajes húmedos, Nov., *Arechavaleta 5101* (lectotype, designated by Parodi (1936), MVM not seen; isolectotype, fragment LP!, type photo, LP!).

Poa patagonica Phil. var. *neuquina* Nicora, Hickenia 1(18): 107. 1977. TYPE: Argentina. Neuquén: Departamento Lácar, San Martín de los Andes, *A. Ruiz Leal 20315* (holotype, BAA!).

Distributed in the northeastern Buenos Aires province, this species reaches its southern limit at the system of Tandilia and Sierras Australes. It extends to the north and east, into southern Entre Ríos, Uruguay, and Brazil. One of its synonyms, *P. patagonica* var. *neuquina,* was found in a Ciprés forest near San Martín de Los Andes, Neuquén, disjunct from its main area of distribution.

Poa denudata Steud., Syn. Pl. Glumac. 1: 259. 1854. TYPE: Chile. Valdivia, *W. Lechler 578* (holotype, LE not seen; pistillate fragment, BAA!; isotype, US not seen).

Poa nahuelhuapiensis Nicora, Hickenia 1(18): 106. 1977. TYPE: Argentina. Neuquén: Departamento Los Lagos, Península Quetrihué, 1 Nov. 1949, *O. Boelcke & J. H. Hunziker 3458* (holotype, BAA!).

Poa denudata grows in western Neuquén, Río Negro, and Chubut, Argentina, and along the Chilean boundary in Regions VIII to X (Toledo & Zapater, 1991), near lake margins, among rocks, or in grasslands below elevations of 1000 m.

Poa lanuginosa Poir., Encycl. 5: 91. 1804. TYPE: Uruguay. Montevideo: *E. Commerson s.n.* (holotype, P not seen; fragment BAA!, US 88769 fragment ex P not seen).

Poa bergii Hieron. var. *chubutensis* Speg., Revista Fac. Agron. Univ. Nac. La Plata 3: 628. 1897. TYPE: Argentina. Chubut: Departamento Florentino Ameghino, Cabo Raso, *C. Spegazzini 938* (holotype, LP not seen; fragment BAA!).

Poa boecheri Parodi, Revista Argent. Agron. 28: 100. 1961 [1962]. TYPE: Argentina. Mendoza: Departamento San Rafael, Valle del Atuel, El Sosneado, 35° lat. S, 4 Oct. 1955, *T. W. Böcher, J. P. Hjerting & K. Rahn 801* (holotype, BAA!; isotype, C not seen).

Poa patagonica Phil., Anales Univ. Chile 94: 168. 1896. TYPE: Chile. Departamento Ultima Esperanza, Lago Pinto, 11 dic. 1877, *H. Ibar s.n.* (holotype, SGO not seen; fragment BAA!, US 88748 fragment ex SGO not seen).

Poa lanuginosa is a widespread species, distributed in Argentina from 35°S to the steppes in Tierra del Fuego. It grows in highly degraded soils, and on dunes and sandy soils in Patagonia.

Poa pogonantha (Franch.) Parodi, Revista Argent. Agron. 20: 180. 1953. *Festuca pogonantha* Franch., Miss. Sci. Cape Horn, Bot. 5: 387. 1889. TYPE: Argentina. Patagonia, Port Eden, 24 Jan. 1879, *Savatier 1844* (holotype, P not seen, fragment ex P, BAA!; isotype, US s.n. fragment and photo ex P not seen).

Poa prichardii Rendle, J. Bot. 42: 324. 1904. TYPE: Argentina. Santa Cruz: Lago Argentino, Monte Buenos Aires, 1900–1, *H. Prichard s.n.* (holotype, BM not seen; fragment BAA!).

This species occurs in humid forests of Río Negro, Chubut, Santa Cruz, and Tierra del Fuego, Argentina (Nicora, 1978), and in the XII Region of Chile (Toledo & Zapater, 1991). This area is included within the Subantarctic phytogeographic province (Cabrera & Willink, 1973; Cabrera, 1994).

Poa rigidifolia Steud. var. **rigidifolia,** Syn. Pl. Glumac. 1: 260. 1854. TYPE: Argentina. Tierra del Fuego e Is. del Atlántico Sur: Islas Malvinas, Is. Soledad, Pto. William, Sep. 1850, *W. Lechler s.n.* (holotype, P not seen; fragment BAA!, US 88734 fragment not seen).

Due to the results obtained in this research, the *P. rigidifolia* complex is considered a single taxonomic species. Two varieties are proposed in agreement with the previous study of Giussani et al. (1996). Pistillate individuals of each variety showed a different pattern of variation to that present in staminate specimens, although correlation between morphology and environment was high for both pistillate and staminate plants. *Poa rigidifolia* var. *rigidifolia* includes *P. spiciformis* (Steud.) Hauman & Parodi (= *P. poecila* Phil., Giussani, 1993), previously synonymized by Giussani and Collantes (1997). It is distinguished by the woolly hairs on the floret callus but an absence of hairs between the palea and lemma nerves on pistillate florets, thinner blades, longer sheaths, ligules, and blades, and smaller stomata than pistillate specimens of *P. rigidifolia* var. *ibari.*

Poa rigidifolia var. **ibari** (Phil.) Giussani, comb. et stat. nov. Basionym: *Poa ibari* Phil., Anales Univ. Chile 94: 170. 1896. TYPE: Chile. Departamento Ultima Esperanza, Lago Pinto, Jan. 1844, *H. Ibar s.n.* (holotype, SGO not seen; isotype, BAA!).

Poa dusenii Hack., in Dusén, Ark. Bot. 7(2): 8. 1908. TYPE: Argentina. Santa Cruz: Mazaredo portum, 47°41′S, Jan. 1905, *Dusén 5318* (holotype, W not seen; fragment BAA!; isotypes, US 89702 not seen, US 1161178 not seen).

Poa rigidifolia var. *ibari* is characterized by not having woolly callus hairs on the floret callus but having them on, and between, principal palea and lemma nerves of pistillate florets.

Further treatment of morphological and environmental variation within *P. rigidifolia* and a discussion of character assessment can be found in Giussani and Collantes (1997). *Poa rigidifolia* inhabits steppes of Chubut, Santa Cruz, and Tierra del Fuego, and similar environments in neighboring Chile. *Poa rigidifolia* var. *rigidifolia* is present in subhumid austral areas, whereas *P. rigidifolia* var. *ibari* grows in more xeric environments (Giussani et al., 1996; Giussani & Collantes, 1997).

Poa tristigmatica E. Desv., in Gay, Fl. Chil. 6: 419. 1853. SYNTYPES: Chile. Magallanes, Cordillera de Talcarengue, Feb. 1831, *Gay 49* (P not seen; fragment, BAA!, US 88717 fragment not seen); Bahía Duclos, Estrecho de Magallanes, *Commerson s.n.* (P not seen).

Poa boelckei Nicora, Hickenia 1(18): 104. 1977. TYPE: Argentina. Neuquén: Departamento Lácar, Chapelco, encima del refugio, 1800–1870 m, 23 Feb. 1974, *M. N. Correa et al. 5926* (holotype, BAB!).

Poa tristigmatica is found in Chile and in Argentina from Mendoza to Tierra del Fuego. Its distributional area falls within the Altoandina phytogeographic province (Cabrera & Willink, 1973; Cabrera, 1994), ranging from 1600 to 2000 m in Neuquén, and at 500 m, or higher, at the highest-latitude areas of Tierra del Fuego. *Poa boelckei* was collected only in Cerro Chapelco at approximately 1800 m.

For the remaining *P. dolichophylla* and *P. resinulosa* complexes, species show allopatric distribution, and a study of population variability and their ecological relationship is required to resolve taxonomic problems. In addition, types of older named taxa, particularly those of Philippi, need to be studied. For this reason, synonymy has not yet been established.

However, the *P. dolichophylla* complex is well segregated from other species within *Poa* sect. *Dioicopoa.* Species distributions are clearly distinct biogeographically. *Poa dolichophylla* and *P. pilcomayensis* var. *calamagrostoidea* inhabit Sistema de Cumbres Calchaquíes-Aconquija-Famatina in northwestern Argentina (González Bonorino, 1958). *Poa stuckertii* is restricted to central Argentina, on hills of Córdoba and San Luis, reaching 1500 m. This taxon is associated with lowland humid soils. *Poa iridifolia* occurs in the Sierras Australes and Tandilia, in Buenos Aires, Argentina (González Bonorino, 1958).

Species of the *P. resinulosa* complex are morphologically similar but inhabit disjunct areas. *Poa resinulosa* grows in central Argentina, between 30° and 45°S, in steppes, on stony, dry soils (Nicora, 1978), and in the Sierras de Tandil and Ventana, in Buenos Aires. *Poa pedersenii* was only collected in Yhu, Paraguay. *Poa calchaquiensis* grows in the Argentinian Puna (the southern extreme of the Peruvian–Bolivian Altiplano) in Jujuy, Salta, Tucumán, and La Rioja and could also be spread over Catamarca. *Poa buchtienii* is distributed on the Altiplano and the Cordillera Oriental of Bolivia.

Morphological variation of both complexes must be addressed by populational and biological studies

to better resolve the degree of similarities and taxonomic boundaries among these species complexes within *Poa* sect. *Dioicopoa*.

Multivariate Key: Classification of the Dioecious Species of Argentina

Diagnostic characters for taxa were selected according to the results of univariate, bivariate and multivariate analyses to generate an identification key. Because most diagnostic characters are quantitative, and ranges of variation usually overlap among entities, this key is based on suites of correlated characters, increasing the accuracy of identification.

Thus, this multivariate key is not a conventional key. It presents several measures for character variation: the frequent range of variation and, between parentheses, the average or mode value (for quantitative or discrete variables, respectively) as well as the minimum and maximum values found within the group. Species identification must be principally guided through the frequent range of variation (numbers in bold); the average or mode shows the most representative value for a species or sample; the minimum and maximum values indicate the largest range observed.

To identify dioecious species of *Poa*, it is desirable to have in hand both pistillate (Pi) and staminate (St) specimens of the population examined. When only a pistillate specimen is available, gynodioecious species from *Poa* subg. *Andinae* Nicora cannot be excluded.

1. Plants **45–80 cm tall** (64 (31–136)); leaves **35–70 cm long** (46.7 (14–93)); blades **20–40 cm long** (28.2 (8–61)); sheaths **12–28 cm long** (18.4 (5.5–39)); panicles **13–25 cm long** (18.4 (6–37)); nodes per panicle, **13–18** (16 (10–20)) 2

1′. Plants **15–55 cm tall** (32.4 (4.3–75)); leaves **5–35 cm long** (20.5 (3.5–54.5)); blades **5–20 cm long** (12.8 (1.3–37)); sheaths **4–12 cm long** (7.6 (1.7–22)); panicles **5–13 cm long** (8.6 (1.7–29)); nodes per panicle, **9–13** (11 (6–20)) 6

2(1). Ligules truncate to obtuse, **0.6–2 mm long** (1.2 (0.4–4.5)); panicle **2.5–6 cm wide** (4 (0.8–10)); abaxial sclerenchyma girders, **1–3** (5) 3

2′. Ligules acuminate, **6–12 mm long** (6.6 (4.5–19.6)); panicle **1.5–3 cm wide** (2.5 (1–4.5)); abaxial sclerenchyma girders: **3–7** 4

3(2). Plants caespitose, iridaceous, laterally compressed with coriaceous sheaths, and wide open panicles at maturity. Leaf blades flat and carinate, **3.5–8 mm wide** (6.6 (1.8–13)). Glume I, **1-nerved; pistillate (Pi) and staminate (St) glumes 2.5–4 mm long** (3.2 (1.4–5.2)), **0.4–0.7 mm wide** (0.6 (0.3–0.8)). Pistillate and staminate lemmas **3–4.5 mm long** (4 (2.5–5)), **0.7–0.9 mm wide** (0.8 (0.6–1.1)). Pistillate and staminate paleas **2.5–3.5 mm long** (3 (2–3.8)), **0.45–0.65 mm wide** (0.5 (0.35–0.8)). Keel and marginal nerve hairs of pistillate florets, when present, less than 0.5 mm long. Sclerenchyma girders at both sides of each vascular bundle: **15–35** (23 (6–45)); sclerenchyma cell groups at blade margin, extending on abaxial and adaxial epidermis. Stomata infrequent on abaxial epidermis, **0.03–0.037 mm long** (0.034 (0.027–0.042)) ..... *Poa dolichophylla* complex

3′. Plants rhizomatous, not laterally compressed, with herbaceous sheaths, panicles contracted to somewhat open at maturity. Leaf blades conduplicate, **2–3.5 mm wide** (2.7 (1.2–4)). Glume I, **1–3-nerved, Pi: 3.7–5 mm long** (4.3 (3.4–5.4)), **St: 2.8–3.7 mm long** (3.2 (2.6–4)); **Pi and St, 0.6–0.8 mm wide** (0.7 (0.6–0.9)). Lemmas, **Pi: 4.5–6 mm long** (5.3 (4–6.7)), **St: 3.8–4.2 mm long** (4 (3.7–4.5)); **Pi and St, 0.85–1.1 mm wide** (1 (0.8–1.3)). Paleas, **Pi: 3.4–4.2 mm long** (3.8 (3–4.6)), **St: 3.1–3.6 mm long** (3.4 (3–4)); **Pi and St: 0.6–0.7 mm wide** (0.65 (0.5–0.9)). Keel and marginal nerve hairs of pistillate florets more than 0.5 mm long. Sclerenchyma girders at both sides of each vascular bundle: **6–12** (9 (5–15)); sclerenchyma cell groups at blade margin, not extending on abaxial and adaxial epidermis. Stomata infrequent on adaxial epidermis. Stomata frequent on adaxial epidermis, **0.042–0.050 mm long** (0.047 (0.037–0.054)) ..... *Poa bonariensis*

4(2). Plants with long and deep rhizomes. Pistillate and staminate spikelets **5- to 6-flowered, 5.5–11 mm long** (7.9 (4.3–12)), **3–8 mm wide** (5.1 (2.2–10)). Glume I, **3–5-nerved**; **Pi: 4–9 mm long** (6.5 (3.6–10)), **St: 3–6 mm long** (4.4 (2.8–7)); **Pi and St: 0.7–1.45 mm wide** (1 (0.6–1.7)). Lemmas, **Pi: 5–9 mm long** (6.9 (4.2–10), **St: 4–7 mm long** (5.4 (3.9–8)); **Pi and St: 0.9–1.6 mm wide** (1.3 (0.7–2.2)). Paleas **Pi: 4–6 mm long** (4.8 (3.3–6.7)), **St: 3–5.3 mm long** (4.2 (3–6.3)); **Pi and St: 0.8–1.3 mm wide** (0.96 (0.5–1.8)) 5

4′. Plants caespitous. Pistillate and staminate spikelets **3-flowered**; **Pi: 4.5–6 mm long** (St: 4 mm); **Pi and St: 1.6–3.5 mm wide.** Glume I, **3-nerved, Pi: 3.5–4 mm long, St: 2.5 mm long; Pi: 0.7–0.8 mm wide** (St: 0.5 mm wide). Lemmas, **Pi: 4–5 mm long, St: 3 mm long; Pi and St: 0.9–1.1 mm wide.** Paleas, **Pi: 3.5–4 mm long, St: 3 mm long; Pi: 0.6–0.8 mm wide, St: 0.5 mm wide.** Restricted to Zapala, Neuquén, Argentina ..... *Poa indigesta*

5(4). Pistillate and staminate spikelets **5.5–8.5 mm long** (6.8 (4.3–9.6)), **3–5.5 mm wide** (4.2 (2.2–6.3)). Glume I, **3-nerved**; **Pi: 4–6 mm long** (5 (3.6–6.8)), **St: 3–5 mm long** (3.8 (2.8–5)); **Pi: 0.8–1.1 mm wide** (0.95 (0.67–1.23)), **St: 0.7–0.9 mm wide** (0.82 (0.6–1.15)). Lemmas, **Pi: 5–7 mm long** (5.8 (4.2–7.5)), **St: 4–5.5 mm long** (4.7 (3.9–5.8)); **Pi and St: 0.9–1.2 mm wide** (1.2 (0.7–2.2)). Paleas, **Pi: 4–5 mm long** (4.2 (3.3–5.1)), **St: 3–4 mm long** (3.6 (3–5.2)); **Pi and St: 0.8–0.9 mm wide** (0.83 (0.5–1.4)). Plants of sandy soils of semiarid Patagonia and Cuyo, Argentina ... *Poa lanuginosa*

5′. Pistillate and staminate spikelets **8–11 mm**

long (9 (6–12)), **3.5–8 mm wide** (6 (3–10)). Glume I, **3–5** (7)**-nerved, Pi: 6.5–9 mm long** (8 (6–10)), **St: 4–6 mm long** (5 (4–7)); **Pi: 1.1–1.45 mm wide** (1.3 (1–1.7)), **St: 0.8–1.1 mm wide** (1 (0.7–1.4)). Lemmas, **Pi: 7–9 mm long** (8 (6–10)), **St: 5–7 mm long** (6 (4.5–8)); **Pi and St: 1.1–1.6 mm wide** (1.4 (1–2)). Paleas, **Pi: 4.6–6 mm long** (5.4 (4–6.7)), **St: 3.6–5.3 mm long** (4.7 (3.3–6.3)); **Pi: 0.9–1.3 mm wide** (1.1 (0.7–1.8)), **St: 0.6–1.1 mm wide** (0.9 (0.6–1.7)). Plants of coastal dunes of Buenos Aires, Argentina ..... *Poa bergii*

6(1). Ligules acuminate, **3–8 mm long** (5.7 (1–16)) 7

6′. Ligules truncate to obtuse, **0.5–2 mm long** (1.5 (0.2–5.8)). [Only *P. denudata* and *P. pogonantha,* rhizomatous species, present ligules 2 to 3 mm long.] 15

7(6). Plants with long and deep rhizomes. Panicles contracted and interrupted, lower branches shorter than first panicle internode; **15–25 cm long** (20.5 (13–29)). Leaf blades subconvolute; **0.14–0.21 mm thick** (0.17 (0.12–0.24)). Bulliform cells not differentiated, abaxial epidermal prickles long, numerous. Lemma **bilobed,** pistillate floret callus with abundant long and rigid hairs; keel, marginal nerves, and intercostal epidermis zones glabrous. Staminate floret glabrous. This species was found only once in dunes of Monte Hermoso and Bahía Blanca, Argentina ..... *Poa schizantha*

7′. Plants caespitous or long-rhizomatous. Panicles spiciform, dense, lower branches as long as or longer than first panicle internode, **5–15 cm long** (7.2 (2–20)). Leaf blades conduplicate; **0.15–0.35 mm thick** (0.25 (0.11–0.5)). Bulliform cells well differentiated; abaxial epidermal prickles frequent, short. Lemma **lanceolate,** not bilobed; pistillate floret callus glabrous or with woolly and long hairs; keel, marginal nerves, and intercostal epidermis zones glabrous or with hairs 0.5 mm or less. Staminate floret glabrous or scarcely pubescent 8

8(7). Plants caespitous. Glume I, **Pi: 2.7–4 mm long** (3.5 (2.4–4.4)), **St: 2.2–3.2 mm long** (2.75 (2–3.5)); **Pi and St: 0.45–0.8 mm wide** (0.7 (0.4–1.1)). Lemmas, **Pi and St: 3.5–5.5 mm long** (4.4 (3–6.4)); **Pi and St: 0.8–1.1 mm wide** (1 (0.6–1.3)). Paleas, **Pi and St**: **2.3–4 mm long** (3.3 (1.8–4.2)); **Pi and St**: **0.55–0.8 mm wide** (0.65 (0.4–0.9)) 9

8′. Plants caespitous or long-rhizomatous. Glume I, **Pi: 4–8 mm long** (5.6 (3.2–9)), **St: 3–7 mm long** (4.8 (2.8–8.2)); **Pi and St: 0.7–1.2 mm wide** (0.9 (0.5–1.4)). Lemmas, **Pi and St: 5–7 mm long** (6 (4–8.5)); **Pi and St: 1–1.5 mm wide** (1.2 (0.7–2.2)). Paleas, **Pi and St: 3–6 mm long** (4.5 (2.6–6.7)); **Pi and St: 0.7–1.2 mm wide** (0.8 (0.5–1.5)) 10

9(8). Ligules **3–6 mm long** (5 (3–9.3)). Pistillate and staminate florets glabrous. Steppes on plains and mountains from San Juan to Neuquén, between 1000 and 3000 m, Argentina ..... *Poa huecu*

9′. Ligules **5–10 mm long** (7 (2–15)). Pistillate florets markedly hairy; callus with woolly, long hairs; keel, marginal nerves, and intercostal epidermis zones glabrous or with hairs 0.5 mm or less. Staminate floret glabrous or scarcely pubescent. Steppes of semiarid Patagonia and central regions of Argentina ..... *Poa ligularis*

10(8). Leaf blades **2–4 mm wide** (2.8 (1.6–5)). Florets frequently viviparous. Glume I, **Pi: 5.5–8 mm long** (6.2 (3.7–9)), **St: 4.5–6.5 mm long** (5.2 (3.6–8)). Lemmas, **Pi: 6–8 mm long** (7.1 (5.5–9.2)), **St: 4.7–7.5 mm long** (6.2 (4.4–9)). Paleas, **Pi and St: 4–6 mm long** (5 (3.7–6.7)); **Pi and St: 0.7–1.2 mm wide** (0.9 (0.5–1.5)). Plants of Patagonian Andes mountains; also widespread on steppes of Santa Cruz and Tierra del Fuego, Argentina 11

10′. Leaf blades **1–2.5 mm wide** (1.6 (0.8–3)). Florets not viviparous. Glume I, **Pi: 4–6 mm long** (5.1 (3.1–6.8)), **St: 3–5.5 mm long** (4.4 (2.8–6.3)). Lemmas, **Pi: 4.5–6.5 mm long** (6 (4–7.1)), **St: 4–6 mm long** (5 (3.7–7.3)). Paleas, **Pi and St: 3.5–4.5 mm long** (4 (2.6–6)); **Pi and St: 0.65–1 mm wide** (0.75 (0.5–1.4)). Plants of Patagonian steppe and semiarid areas of Cuyo and central Argentina 12

11(10). Ligules **2–5.5 mm long** (3.6 (1.2–9)). Plants **18–38 cm tall** (26 (15–62)); leaves **8–18 cm long** (14 (5.5–30)); leaf blades **5–10 cm long** (8.3 (3–15)), **0.25–0.40 mm thick** (0.33 (0.14–0.5)); sheaths **4–8 cm long** (6 (2.5–16)). Stomata **0.044–0.053 mm long** (0.048 (0.042–0.062)). Glume I, **Pi: 4.5–6.2 mm long** (5.5 (3.7–7)), **St: 4–5.3 mm long** (4.5 (3.6–5.6)). Lemmas, **Pi: 5.8–7.8 mm long** (6.7 (5.5–9)), **St: 4.7–6 mm long** (5.4 (4.4–6.7)). Paleas, **Pi and St: 4–5.5 mm long** (4.7 (3.8–6)). Patagonian Altoandina biogeographic province, Argentina and Chile ..... *Poa tristigmatica*

11′. Ligules **4–8 mm long** (6 (1.5–11)). Plants **25–50 cm tall** (34 (9–73)); leaves **15–35 cm long** (24 (4.5–52)); leaf blades **8–20 cm long** (13.4 (2.2–30)), **0.20–0.28 mm thick** (0.25 (0.17–0.36)); sheaths **6–14 cm long** (10 (2.5–22)). Stomata **0.036–0.046 mm long** (0.042 (0.032–0.052)). Glume I, **Pi: 6–8 mm long** (6.8 (4.8–9)), **St: 4.7–7 mm long** (6 (4.3–8.2)). Lemmas, **Pi: 6.5–8.5 mm long** (7.5 (5.7–9.2)), **St: 5.5–8 mm long** (7 (5–9)). Paleas, **Pi and St: 4.5–6 mm long** (5.2 (4.2–6.7)). Southeast Patagonia and Chile ..... *Poa alopecurus*

12(10). Leaf blades **2–3 mm wide** (2.6 (1.8–4)). Pistillate and staminate florets glabrous. Steppes and mountains from San Juan to Mendoza, between 1900 and 3900 m, Argentina ..... *Poa holciformis*

12′. Leaf blades **1.2–2 mm wide** (1.6 (0.8–3)). Pistillate floret callus with woolly, long hairs; keel, marginal nerves, and intercostal epidermis zones glabrous or with hairs 0.5 mm

or longer; staminate florets glabrous or scarcely pubescent. Steppes of semiarid Patagonia 13

13(12). Plants long-rhizomatous, **20–50 cm tall** (38 (13–61)); panicles **7–14 cm long** (10.3 (4.5–19.8)); panicle nodes: **11–15** (13 (9–18)). Leaves **15–35 cm long** (25 (7.2–47)); leaf blades **7–27 cm long** (17 (4.7–35)) and **0.23–0.34 mm thick** (0.28 (0.18–0.41)); sheaths **4–14 cm long** (8.25 (2.5–20)); ligules **5–11 mm long** (7.8 (3.3–16.4)). Stomata **0.044–0.054 mm long** (0.048 (0.040–0.060)); keel and marginal nerves of pistillate florets with hairs longer than 0.5 mm long, intercostal epidermis zones glabrous. Widespread on semiarid Patagonia, and coasts of Argentina, Brazil, and Uruguay ..... *Poa lanuginosa*

13′. Plants caespitous, **8–25 cm tall** (17.6 (6–42)); panicles **3–7 cm long** (4.4 (1.7–9)); panicle nodes: **7–11** (8 (6–14)). Leaves **5–18 cm long** (10.2 (3.5–36.5)); leaf blades **3–8 cm long** (5.6 (1.3–24)), **0.15–0.23 mm thick** (0.19 (0.11–0.28)); sheaths **2–7 cm long** (4.6 (1.7–14.8)); ligules **3–7 mm long** (4.7 (1.1–10.3)). Stomata **0.033–0.044 mm long** (0.038 (0.030–0.054)); keel and marginal nerves of pistillate florets with hairs shorter than 0.5 mm long, intercostal epidermis zones pubescent or glabrous. Southern Patagonia, Argentina 14

14(13). Callus of pistillate florets hairy, with woolly, long hairs, intercostal epidermis zones of lemma and palea generally glabrous. On pistillate specimens: sheaths **3.5–6.5 cm long,** blades **0.8–0.11 mm thick,** and stomata **0.029–0.038 mm long**; on staminate specimens: spikelets **5.4–7.8 mm long** and leaf blades **1–1.5 mm long.** Subhumid localities of Santa Cruz and Tierra del Fuego, Argentina ..... *Poa rigidifolia* var. *rigidifolia*

14′. Callus of pistillate florets glabrous, intercostal epidermis zones of lemma and palea usually pubescent. On pistillate specimens: sheaths **1.8–4.8 cm long,** blades **0.12–0.16 mm thick;** stomata **0.034–0.042 mm long;** on staminate specimens: spikelets **4.7–6.5 mm long** and leaf blades **0.8–1.3 mm long.** Xeric localities of Santa Cruz and Chubut, Argentina ..... *Poa rigidifolia* var. *ibari*

15(6). Florets not viviparous. Glume I, **Pi: 2.5–4.5 mm long** (3.4 (1.8–4.8)), **St: 2–3.5 mm long** (2.7 (1.5–3.8)); **Pi and St: 0.45–0.8 mm wide** (0.58 (0.33–1)). Lemmas, **Pi: 3–5.5 mm long** (4.5 (2.8–6)), **St: 3–4 mm long** (3.5 (2.8–4.1)); **Pi and St: 0.6–1 mm wide** (0.78 (0.54–1.2)). Paleas, **Pi and St: 2.8–3.8 mm long** (3.1 (1.8–4.1)); **Pi and St: 0.4–0.7 mm wide** (0.54 (0.3–1)) 16

15′. Florets usually viviparous [not in *P. hubbardiana,* infrequently in *P. denudata*]. Glume I, **Pi: 4–7 mm long** (5.4 (3.3–7.5)), **St: 3.5–6.5 mm long** (4.5 (2.9–7.5)); **Pi and St: 0.7–1.1 mm wide** (0.82 (0.52–1.3)). Lemmas, **Pi: 4.5–8 mm long** (6.3 (5–8.8)), **St: 4–6.5 mm long** (5.1 (4–7)); **Pi and St: 0.9–1.3 mm wide** (1.1 (0.72–1.5)). Paleas, **Pi and St: 3.5–5 mm long** (4.1 (2.6–5.3)); **Pi and St: 0.7–0.8 mm wide** (0.75 (0.5–1)) 17

16(15). Plants caespitous or long-rhizomatous. Leaf blades **1–2 mm wide** (1.6 (0.9–3.2)). Stomata **0.032–0.040 mm long** (0.037 (0.027–0.046)). Spikelets, **Pi: 4–6.5 mm long** (5.6 (3.3–7.2)), **St: 4–5 mm long** (4.6 (3.4–5.4)). Glume I, **Pi: 2.5–3.5 mm long** (3 (1.8–4.8)), **St: 2–3 mm long** (2.5 (1.5–3.3)); **Pi and St: 0.4–0.7 mm wide** (0.54 (0.33–1)). Lemmas, **Pi: 3–5 mm long** (4.1 (2.8–5.4)), **St: 2.8–3.5 mm long** (3.4 (2.8–4.1)). Paleas, **Pi and St: 2.5–3.5 mm long** (2.9 (1.8–3.7)) 18

16′. Plants caespitous. Leaf blades **2–4 mm wide** (3.1 (2–4.4)). Stomata **0.037–0.044 mm long** (0.040 (0.036–0.046)). Spikelets, **Pi: 6.4–7 mm long** (6.8 mm (6.3–7.2)), **St: 4.5–6 mm long** (5.6 (4.8–6.3)). Glume I, **Pi: 3.5–4.5 mm long** (4.2 (3.8–4.6)), **St: 2.8–3.5 mm long** (3.2 (2.8–3.8)); **Pi and St: 0.5–0.8 mm wide** (0.67 (0.40–0.9)). Lemmas, **Pi: 4.8–5.8 mm long** (5.3 (4.7–6)), **St: 3.5–4 mm long** (3.8 (3.2–4.1)). Paleas, **Pi: 3.2–4 mm long** (3.6 (3.1–4.1)), **St: 2.8–3.5 mm long** (3.2 (2.8–3.8)) ..... *Poa lanigera*

17(15). Plants caespitous. Inflorescences usually not longer than leaf length. Ligules obtuse, **0.6–1 mm long** (1.1 (0.5–1.9)). Stomata scarce on abaxial epidermis, **0.33–0.040 mm long** (0.036 (0.027–0.045)). Leaf blades with apex acuminate to acute. Florets not viviparous. Pistillate floret hairy, cottonlike; callus with woolly, long hairs; keel, marginal nerves, and intercostal epidermis zones with hairs over 1 mm long. Staminate florets glabrous or with a few long, woolly hairs on callus. Grasslands above 1500 m in Córdoba and San Luis hills, Argentina ..... *Poa hubbardiana*

17′. Plants rhizomatous. Inflorescences longer than the leaf length. Ligules acuminate, **1–4 mm long** (2.2 (0.5–5.8)). Stomata frequent on the abaxial epidermis, **0.035–0.045 mm long** (0.042 (0.034–0.055)). Leaf blades with apex navicular to obtuse. Florets usually viviparous. Pistillate floret callus without hairs, or with short or long, woolly hairs; keel, marginal nerves, and intercostal epidermis zones glabrous, or with hairs shorter than 0.5 mm. Staminate florets glabrous or scarcely pubescent. Patagonian Andes mountains, up to 1000 m 19

18(16). Plants caespitous or long-rhizomatous. Leaf blades conduplicate; **0.15–0.24 mm thick** (0.20 (0.17–0.48)). Glume I, **1-nerved.** Lemmas, **Pi and St: 0.7–0.9 mm wide** (0.8 (0.65–1.2)). Paleas, **Pi and St: 0.45–0.7 mm wide** (0.6 (0.4–1)) ..... *Poa resinulosa* complex

18′. Plants caespitous. Leaf blades flat; **0.13–0.17 mm thick** (0.15 (0.13–0.48)). Glume I, **3-nerved** (1). Lemmas, **Pi and St: 0.6–0.7 mm wide** (0.65 (0.54–0.86)). Paleas, **Pi**

and St: 0.35–0.45 mm wide (0.4 (0.3–0.7)) ..... *Poa pilcomayensis* var. *pilcomayensis*

19(17). Florets usually viviparous. Spikelets, **Pi and St: 7–20 mm long** (14 (5.6–28.6)). Glume I, **Pi: 5–6.8 mm long** (6 (4.3–7.1)), **St: 4–6.5 mm long** (5.6 (3.9–7.5)); **Pi and St: 0.7–1 mm wide** (0.90 (0.64–1.3)). Lemmas, **Pi: 5.5–8 mm** (7 (5–8.8)), **St: 5–6.5 mm** (6 (4.8–6.9)). Pistillate floret callus with short or long, woolly hairs ..... *Poa pogonantha*

19'. Viviparous florets absent, occasionally present. Spikelets, **Pi and St: 5–7 mm long** (6 (5–7.7)). Glume I, **Pi: 3.5–5 mm long** (4.3 (3.3–5.3)), **St: 3–4 mm long** (3.6 (3–4.1)); **Pi and St: 0.6–0.8 mm wide** (0.72 (0.51–0.83)). Lemmas, **Pi: 4.5–5.5 mm long** (5.3 (4.3–5.8)), **St: 4–5 mm long** (4.5 (4–5.2)). Pistillate floret callus with long and woolly hairs ..... *Poa denudata*

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Appendix 1.

List of species. Letters in parentheses correspond to the abbreviation of the species name used in the phenetic analysis.

Poa alopecurus (Gaudich.) Kunth (AL)
P. barrosiana Parodi (BA)
P. bergii Hieron. (BE)
P. bergii var. *chubutensis* Speg. (BU)
P. boecheri Parodi (BO)
P. boelckei Nicora (BK)
P. bonariensis (Lam.) Kunth (BN)
P. buchtienii Hack. (BT)
P. calchaquiensis Hack. (CL)
P. denudata Steud. (DE)
P. dolichophylla Hack. (DO)
P. dusenii Hack. (DU)
P. holciformis J. Presl (HO)
P. hubbardiana Parodi (HB)
P. huecu Parodi (HU)
P. ibari Phil. (IB)
P. indigesta Parodi (IN)
P. iridifolia Hauman (IR)
P. lanigera Nees (LG)
P. lanuginosa Poir. (LA)
P. ligularis Nees ex Steud. (LI)
P. montevidensis Arechav. (MO)
P. nahuelhuapiensis Nicora (NA)
P. patagonica Phil. (PA)
P. patagonica var. *neuquina* Nicora (PN)
P. pedersenii Nicora (PE)
P. pilcomayensis Hack. (PI)
P. pilcomayensis var. *calamagrostoidea* Hack. (PC)
P. pogonantha (Franch.) Parodi (PG)
P. prichardii Rendle (PR)
P. resinulosa Nees ex Steud. (RE)
P. rigidifolia Steud. (RI)
P. schizantha Parodi (SC)
P. shuka (Speg.) Parodi (SK)
P. stuckertii (Hack.) Parodi (ST)
P. superbiens (Steud.) Hauman & Parodi (SU)
P. tristigmatica E. Desv. ex Gay (TR)

Appendix 2.

Specimens listed here were used as OTUs in the phenetic analysis. They are sorted alphabetically by species or species complexes as identified in this research. Previous specimen determination is indicated between brackets corresponding to the species abbreviation as set in the list of species. Pistillate specimens "Pi" or staminate specimens "St" are also indicated between brackets. Islas Malvinas represents a similar location to the Falkland Islands on specimen labels.

Poa alopecurus

ARGENTINA. **Santa Cruz:** Depto. Güer Aike, Entre Glencross y Pta. Alta, 51°46′S, 71°43′W, 1976, *Latour et al. 1004* (BAB) [AL, Pi and St]; Barranca al norte de 28 de Noviembre, 51°35′S, 72°11′W, 13 Jan. 1976, *Latour et al. 382* (BAB) [SK, Pi]; ruta 3, a 8 km al W de Río Gallegos, 51°37′S, 69°25′W, 5 Nov. 1977, *Roig & Méndez 2361* (BAB) [SK, Pi and St]; Estancia Cerro Castillo, Laguna Figueroa, 51°28′S, 72°27′W, 12 Jan. 1977, *Latour et al. 1367* (BAB) [SU, St.]; Lago Argentino, Puerto Bandera, 50°18′S, 72°47′W, *Correa et al. 3032* (BAA) [SU, Pi]; Estancia Las Viscachas, Cerro Las Viscachas, 50°46′S, 72°01′W, 25 Jan. 1977, *Arroyo et al. 2428* (BAB) [SU, Pi]; Cerro sin nombre, 50°46′S, 72°08′W, 29 Jan. 1977, *Arroyo et al. 2643* (BAB) [SU, Pi]. **Tierra del Fuego e Isla del Atlántico Sur:** Islas Malvinas, Isla Soledad, 52°00′S, 59°00′W, *Gaudichaud C. s.n.* (*Arundo alopecurus* Gaudich. ex Mirb., syntype, BAA, fragment) [AL, Pi]; Islas Malvinas, 52°00′S, 59°00′W, *Hooker s.n.* (*Festuca antarctica* var. γ Hook. f., isotype, BAA fragment) [AL, Pi and St]; Ushuaia, Is. de los Estados, Port Vancouver, 54°49′S, 64°20′W, *Spegazzini s.n.* (*Festuca shuka* Speg., isotype, LP) [SK, Pi]; Canal de Beagle, Isla de los Gaviotines, 54°52′S, 68°18′W, *Vervoorst 161* (BAA) [AL,

St]; Islas de los Estados, Bahía Liberty, 54°50′S, 64°25′W, 2 Nov. 1971, *Dudley et al. 1301* (BAB) [AL, St]; Islas de los Estados, Puerto Vancouver, 54°49′S, 64°20′W, 16 Jan. 1934, *Castellanos 12868* (BAB) [AL, St]; Islas de los Estados, Puerto Cook, 54°50′S, 64°20′W, 4 Jan. 1934, *Castellanos 12829* (BAB) [AL, St]; Is. de los Estados, Pto. Cook, 54°49′S, 64°20′W, 4 Jan. 1934, *Castellanos 12829b* (SI) [SK, Pi]; Bahia Thetis, Región del Río del Fuego, 54°12′S, 67°23′W, Mar. 1902, *Holmberg & Calcagnini 3604* (BAB) [AL, Pi]; Ea. Harberton, Campo Rancho Tambo, shore of Bahía Cambaceres, 54°52′S, 67°16′W, 8 Jan. 1968, *Moore 1373* (BAB) [AL, Pi]; Bahía Agüirre, Puerto Español, E end beyond settlement Ostoic, 54°53′S, 65°58′W, 19 Feb. 1968, *Moore 1875* (BAB) [AL, St]; Remolino, 54°50′S, 67°53′W, 21 Dec. 1932, *Castellanos 7579* (BAB) [AL, St]. CHILE. **XII Región:** Sandy Point, 53°10′S, 70°54′W, *W. Lechler 1194* (*Aira superbiens* Steud., isotype, BAA fragment) [SU, Pi]; Islote Pto. Luisa, 54°56′S, 67°38′W, *Roig et al. 2838* (BAA,) [AL, St]; Sierra Baguales, Ea. La Cumbre, Co. sin nombre, 50°42′S, 72°22′W, 18 Dec. 1975, *Boelcke et al. 682* (BAB) [AL, Pi and St]; Punto Bella Vista, 51°30′S, 73°15′W, 5 Dec. 1979, *Roig et al. 5097* (BAB) [AL, Pi]; Estancia Cerro Castillo, Lago Sofía, 51°32′S, 72°37′W, 14 Jan. 1977, *Latour et al. 1493* (BAB) [SK, Pt]; Laguna Figueroa, 51°28′S, 72°27′W, 12 Jan. 1977, *Latour et al. 1367* (BAB) [SU, St.]; Sección Tres Pasos, Hotel, 51°25′S, 72°29′W, 14 Jan. 1977, *Latour et al. 1600* (BAB) [SU, Pi]; *Latour et al. 1574* (BAB) [SU, Pi].

Poa bergii

ARGENTINA. **Buenos Aires:** Bahía Blanca, Punta Alta, 38°53′S, 62°04′W, Feb. 1902, *Spegazzini et al. 2381* (BAB) [BE, Pi]; Partido General Dorrego, Monte Hermoso, 38°59′S, 61°17′W, 30 Oct. 1985, *Villamil 3393* (SI) [BE, Pi and St], 31 Oct. 1985, *Villamil 3413* (SI) [BE, Pi], 9 Nov. 1986, *Villamil 4291* (SI) [BE, Pi]; Camino a Monte Hermoso, Balneario Sauce Grande, 38°59′S, 61°17′W, 31 Oct. 1986, *Villamil 3432* (SI) [BE, Pi], 30 Oct. 1965, *Cabrera et al. 17061* (LP) [BE, Pi], *Cabrera et al. 17063* (LP) [BE, St], *Cabrera et al. 17065 1/2* (LP) [BE, St], 24 Nov. 1963, *Fabris & Schwabe 4821* (LP) [BE, Pi]; Partido Pellegrini, Pellegrini, 36°16′S, 63°10′W, 28 Nov. 1940, *Cabrera 6948* (LP), [BE, Pi]; Partido Coronel Rosales, Pehuen-Co, 39°00′S, 61°33′W, 19 Nov. 1962, *Cabrera & Fabris 14914* (LP) [BE, Pi]; Partido Villarino, Médanos, 38°50′S, 62°42′W, 6 Nov. 1940, *Cabrera 6673* (LP) [BE, Pi]; Partido General Madariaga, Pinamar, 37°07′S, 56°51′W, 11 Dec. 1950, *Cabrera 10707* (LP) [BE, Pi]; Partido Tres Arroyos, Claromecó, 38°52′S, 60°04′W, 8 Nov. 1986, *Villamil 4211* (SI) [BE, Pi]; Partido General Alvarado, Miramar, 38°16′S, 57°52′W, 31 Jan. 1930, *Parodi 9820* (holotype, BAA) [BA, Pi], 11 Nov. 1939, *Cabrera 5555* (BAA) [BA, St], Sep. 1922, *Hauman 5762* (BAA) [BA, Pi]; Partido Lobería, Quequén, 38°32′S, 58°42′W, Jan. 1930, *Cabrera 1317* (SI) [BA, St], Feb. 1925, *Parodi 6398* (BAA) [BA, St], 7 Nov. 1943, *Boelcke s.n.* (SI) [BA, Pi and St]; Partido General Pueyrredón, Mar del Plata, 38°00′S, 57°34′W, 9 Dec. 1947, *Boelcke 2838* (BAA) [BA, Pi], Jan. 1923, *Barros 4898* (BAA) [BA, Pi], 9 Dec. 1947, *Boelcke 2837* (BAA) [BA, St]. **Río Negro:** Adolfo Alsina, médanos en la boca del Río Negro, 41°03′S, 62°48′W, *Berg 205* (holotype, CORD) [BE, Pi]. URUGUAY. **Colonia:** Riachuelo, 34°25′S, 57°52′W, 12 Oct. 1936, *Cabrera 3872* (BAA) [BE, Pi].

Poa bonariensis

ARGENTINA. **Buenos Aires:** Río Viedma, Sep. 1934, *Ruiguelet 409* (BAA) [BN, Pi and St]; Partido Avellaneda, Avellaneda, 34°40′S, 58°23′W, 14 Oct. 1922, *Parodi 4747* (BAA) [BN, Pi and St]; Partido Dolores, Laguna Sevigné, 36°12′S, 57°44′W, 11 Oct. 1962, *Cano 1* (BAA) [BN, St]; Partido La Plata, La Plata, en el Jardín Zoológico, 34°55′S, 57°57′W, Nov. 1962, *Torres 1023* (LP) [MO, St]; Partido Cañuelas, Ruta 3, km 64, entre Brandsen y Monte, 35°03′S, 58°46′W, 13 Nov. 1962, *Cano-Cámara 3* (SI) [MO, Pi]. **Entre Ríos:** Depto. Gualeguaychú, E. Carbó, 33°09′S, 59°14′W, 23 Oct. 1949, *Burkart 18132* (SI) [BN, Pi]; Paranacito, 33°42′S, 59°01′W, Oct. 1949, *Ragonese & Crovetto 25* (BAB) [BN, Pi and St]; Médanos, 33°25′S, 59°04′W, 2 Dec. 1930, *Parodi 9445* (BAA) [BN, Pi and St]; Delta inferior, Arroyo Martínez, 33°52′S, 58°40′W, Oct. 1944, *Burkart 15044* (SI) [MO, St], *Burkart 15043* (SI) [MO, St]; Depto. Gualeguay, La Verde, 33°09′S, 59°20′W, 21 Oct. 1949, *Burkart 18068* (SI) [BN, Pi]; Depto. La Paz, Arroyo Feliciano, 30°44′S, 59°38′W, 17 Oct. 1980, *Muñoz 1353* (SI) [MO, Pi and St]. **Neuquén:** Depto. Lácar, San Martín de Los Andes, 40°10′S, 71°21′W, 30 Jan. 1959, *Ruiz Leal 20315* (holotype, BAA) [PN, Pi], 6 Jan. 1938, *Giacobbi 12961* (BAA) [PN, Pi], 22 Jan. 1966, *Eskuche 591* (BAA) [PN, Pi]; Cerro al Norte de San Martín de los Andes, 40°10′S, 71°21′W, 10 Dec. 1946, *Dawson 1287 1/2* (BAA) [PN, Pi]. URUGUAY. **Montevideo:** Montevideo, 35°00′S, 56°05′W, en parajes húmedos, Nov., *Arechavaleta 5101* (isotype, LP fragment) [MO, Pi and St]; Fray Bentos, 33°10′S, 58°15′W, 5 Oct. 1934, *Meyer 1037* (BAA) [MO, St]. **Soriano:** Arroyo Grande, Paso de Los Loros, 19 Nov. 1937, *Rosengurtt 2294* (BAA) [MO, St].

Poa denudata

ARGENTINA. **Neuquén:** Depto. Picunches, Pino Hachado, Orilla afluente Arroyo Haichol, 38°40′S, 70°54′W, 21 Jan. 1963, *Valla et al. 3024* (BAA) [DE, Pi and St]; Depto. Los Lagos, Villa Puerto Manzano, 40°49′S, 71°37′W, 8 Dec. 1963, *Diem 3190* (BAA) [DE, Pi and St]; Cerro O'Connor, 40°49′S, 71°37′W, 25 Nov. 1963, *Diem 3009* (BAA) [DE, Pi and St]; Isla Victoria, 40°54′S, 71°34′W, 11 Nov. 1946, *Perrone 15212* (BAA) [NA, St]; Depto. Minas, Lago Epu-Lauquen, Arroyo Pincheira, 36°50′S, 71°03′W, 18 Jan. 1964, *Boelcke et al. 11003* (SI) [DE, Pi]; Península Quetrihué, 40°52′S, 71°38′W, 1 Jan. 1949, *Boelcke & Hunziker 3458* (holotype, BAA) [NA, Pi and St]; Puerto San Patricio, 40°52′S, 71°38′W, 7 Nov. 1940, *Diem 285* (BAA) [NA, St], 30 Nov. 1942, *Diem 415* (BAA) [NA, St]. **Río Negro:** Depto. Nahuel Huapi, Lago Gutiérrez, 41°08′S, 71°20′W, 28 Oct. 1949, *Boelcke & Hunziker 3418* (BAA) [DE, Pi and St]. CHILE. **X Región:** Valdivia, 39°49′S, 73°14′W, *Lechler W. 578* (BAA, fragment) [DE, Pi].

Poa dolichophylla complex

Poa dolichophylla: ARGENTINA. **La Rioja:** Depto. Famatina, Sierra Famatina, Mina El Oro, 28°55′S, 67°31′W, 6 Feb. 1956, *Calderón 1115* (BAA) [DO, Pi], 7 Feb. 1956, *Calderón 1168* (BAA) [DO, Pi]. **Tucumán:** Depto. Chicligasta, La Banderita, ruta 65, 27°20′S, 66°00′W, 14 Oct. 1966, *Boelcke et al. 5505* (BAA) [DO, Pi and St]; Depto. Tafí, Bajo de Anfama, 27°46′S, 65°34′W, 6 Aug. 1906, *Lillo 5066* (isotype, BAA) [DO, Pi]; Valle Calchaquí, Peñas Azules, 26°35′S, 65°40′W, 27

Jan. 1933, *Parodi 10926* (BAA) [DO, Pi]; Cerro El Negrito, 26°37′S, 65°44′W, 19 Jan. 1964, *Giusti et al. 3863* (BAA) [DO, Pi]; La Puerta, 29 Jan. 1933, *Parodi 10891* (LP) [DO, St]; Valle Calchaquí, Peñas Azules, 26°35′S, 65°40′W, 27 Jan. 1933, *Parodi 10927* (BAA) [DO, Pi and St]. *P. iridifolia*: ARGENTINA. **Buenos Aires:** Partido Balcarce, Quinta Sagenave, 37°52′S, 58°15′W, 12–25 Oct. 1943, *Hunziker 3930* (BAA) [IR, St]; Partido General Pueyrredón, Mar del Plata, Sierra La Brava, 37°50′S, 57°55′W, 4 Dec. 1930, *Hicken 40* (SI) [IR, Pi]; Partido Saavedra, Sierra de Curamalal, La Gruta, 37°52′S, 62°21′W, 12 Nov. 1938, *Cabrera 4756* (SI) [IR, St]; Partido Tandil, Sierra de las Animas, 37°29′S, 59°18′W, 21 Nov. 1940, *Cabrera 6810* (SI) [IR, Pi]; Tandil, 37°20′S, 59°08′W, 20 Nov. 1929, *Hunziker 8971* (BAA) [IR, St], 15 Nov. 1953, *Burkart 19263* (SI) [IR, Pi]; Sierras de Tandil, 37°20′S, 59°08′W, 1 Nov. 1919, *Hicken 21* (SI) [IR, St]; Partido Tornquist, Sierra de la Ventana, Abra de la Ventana, 38°08′S, 62°00′W, 7 Nov. 1938, *Cabrera 4687* (SI) [IR, Pi]; Cerro de la Ventana, 38°08′S, 61°47′W, 7 Oct. 1939, *Cabrera 5336* (SI) [IR, Pi], 9 Nov. 1938, *Cabrera 4746* (SI) [IR, St]; Cerro Napostá, Estancia Laurina, 38°08′S, 61°47′W, 17 Nov. 1972, *Gómez et al. 11719* (BAA) [IR, Pi]. *P. pilcomayensis* var. *calamagrostidea*: ARGENTINA. **Tucumán:** Depto. Chicligasta, Estancia Las Pavas, Puerto El Bayo, 27°19′S, 65°55′W, Mar. 1924, *Venturi 3077* (SI) [PC, St], *Venturi 3074* (SI) [PC, Pi]; Depto. Famaillá, Quebrada del Río Colorado, 27°10′S, 65°22′W, 26 Aug. 1939, *Meyer 14980* (BAA) [PC, Pi and St]; Quebrada de Lules, 26°56′S, 65°21′W, 5 Nov. 1929, *Venturi 9066* (LP) [PC, St]; Depto. Monteros, Quebrada de los Sosa, 27°09′S, 65°34′W, Dec. 1960, *Burkart 22098* (BAA) [PC, Pi]; Depto. Tafí, Bajo de Anfama, 27°46′S, 65°34′W, 6 Sep. 1906, *Lillo 5064* (isotype, SI fragment) [PC, St]; Puerta de San Javier, 26°46′S, 65°21′W, Dec. 1923, *Venturi 2540* (SI, LP) [PC, Pi and St]; Depto. Trancas, Playa del río de la Hoyada, 26°34′S, 66°24′W, 23 Nov. 1921, *Scheiter 1855* (BAA) [PC, Pi]. *P. stuckertii*: ARGENTINA. **Córdoba:** Depto. Pocho, Camino a los Gigantes, Cerro Blanco, próximo al Río Juspe, 31°27′S, 64°38′W, 5 Dec. 1958, *Nicora 6642* (BAA) [ST, Pi]; Depto. San Alberto, Pampa de Achala, 31°36′S, 64°45′W, 10 Nov. 1925, *Hicken 16565* (BAA) [ST, St], 1–4 Dec. 1926, *Parodi 7582* (BAA) [ST, Pi], *Parodi 7621* (BAA) [ST, Pi and St]; La Posta, 31°36′S, 64°45′W, 7 Dec. 1958, *Burkart 20901* (SI) [ST, St], 9 Dec. 1995, *Giussani stm2* (SI) [ST, St], *Giussani stm6* (SI) [ST, St]. **San Luis:** Depto. Chacabuco, Sierra Comechingones, 32°54′S, 65°04′W, 15 Nov. 1925, *Castellanos s.n.* (BAA) [ST, Pi]; Depto. Pringles, La Carolina, 32°49′S, 66°06′W, 8 Nov. 1940, *Burkart 10787* (BAA) [ST, Pi].

Poa holciformis

ARGENTINA. **Mendoza:** Depto. Las Heras, Las Cuevas, Refugio Militar General Lamadrid, 32°49′S, 70°03′W, 10 Jan. 1963, *Boelcke et al. 9720* (BAB, SI) [HO, Pi and St]; Depto. San Carlos, Los Paramillos, camino a Laguna Diamante, 34°10′S, 69°35′W, 23 Jan. 1989, *Gómez-Sosa 345* (SI) [HO, Pi]; 6 km W Refugio Militar General Alvarado, 34°10′S, 69°45′W, 17 Jan. 1963, *Boelcke et al. 9983* (BAB) [HO, Pi]. **Neuquén:** Depto. Chos Malal Cajón del Arroyo del cruce, 36°43′S, 70°23′W, 27 Jan. 1964, *Boelcke et al. 11265* (BAB) [HO, Pi]; Vegas del Pelán, camino a Riscos Bayos, 36°54′S, 70°20′W, 24 Jan. 1964, *Boelcke et al. 11157* (BAA) [HO, Pi and St]; Riscos Bayos, confluencia Arroyo Olletas con el Arroyo Curileuvú, 36°57′S, 70°20′W, 25 Jan. 1964, *Boelcke et al. 11175* (BAA, BAB) [HO, Pi]; Depto. Minas, Sierra de Cochicó, 36°21′S, 70°34′W, 29 Jan. 1970, *Boelcke et al. 14087* (BAA) [HO, St]; Nacimiento de la cordillera del Viento, 36°46′S, 70°31′W, 2 Feb. 1964, *Boelcke et al. 11540* (BAB) [HO, Pi and St]. CHILE. In Cordillera chilensibus, *Haenke s.n.* (isotype, BAA fragment) [HO, Pi].

Poa hubbardiana

ARGENTINA. **Córdoba:** Depto. San Alberto, Sierra de Achala, al bajar de la Pampa de Achala, 31°36′S, 64°45′W, 1–4 Dec. 1926, *Parodi 7501* (holotype, BAA) [HB, Pi]; Sierra de Achala, 31°36′S, 64°45′W, 15 Nov. 1878, *Hieronymus s.n.* (BAA) [HB, Pi and St]; Pampa de Achala, La Posta, 31°36′S, 64°45′W, 9 Dec. 1995, *Giussani s.n.* (SI) [HB, St]. **San Luis:** Depto. Chacabuco, Sierra de Comechingones, 32°54′S, 65°04′W, 23 Nov. 1925, *Castellanos s.n.* (BAA) [HB, St]; Depto. La Capital, Ciudad, 33°18′S, 66°22′W, *Parodi 2615* (BAA) [HB, St]; Depto. Pringles, La Carolina, 32°49′S, 66°06′W, 8 Nov. 1940, *Burkart 10792* (SI) [HB, Pi and St]; *Burkart 10781* (SI) [HB, Pi].

Poa huecu

ARGENTINA. **Mendoza:** Depto. Las Heras, Puente del Inca, Valle de los Horcones, 32°50′S, 69°55′W, 12 Jan. 1963, *Boelcke et al. 9818* (BAB) [HU, St]. **Neuquén:** Depto. Chos Malal, Cajón Grande, Cordillera del Viento, 36°58′S, 70°30′W, 25 Jan. 1935, *Ragonese A. 284A* (holotype, BAA) [HU, Pi]; Cajón de Atreuco, Cordillera del Viento, 36°58′S, 70°30′W, 25 Jan. 1935, *Ragonese 284* (BAA) [HU, St]; Cajón inferior del Arroyo Turbio llamado localmente Arroyo Domuyo, 36°44′S, 70°23′W, 28 Jan. 1964, *Boelcke et al. 11323* (BAA) [HU, Pi]; Depto. Minas, Laguna Varvarco Campos, Arroyo Enfermera curso inferior, 36°17′S, 70°39′W, 29 Jan. 1970, *Boelcke et al. 14061* (BAB) [HU, St]; Arroyo Enfermera, extremo sur, 36°23′S, 70°37′W, 28 Jan. 1970, *Boelcke et al. 14036* (BAB) [HU, Pi]; Baños Calientes, Río Varvarco, 36°42′S, 70°37′W, 31 Jan. 1964, *Boelcke et al. 11424* (BAB) [HU, Pi]; Depto. Zapala, Zapala, 38°55′S, 70°04′W, *Hicken 16564* (SI) [HU, Pi]; El Sauce, 39°02′S, 70°00′W, 11 Dec. 1952, *Cabrera 11175* (LP) [HU, Pi].

Poa indigesta

ARGENTINA. **Neuquén:** Depto. Zapala, Zapala, 38°55′S, 70°04′W, 7 Dec. 1946, *Dawson G. 1227* (holotype, BAA) [IN, Pi and St], 5 Feb. 1920, *Parodi 2721* (BAA) [IN, Pi].

Poa lanigera

ARGENTINA. **Buenos Aires:** Partido Pehuajó, Pehuajó, 35°48′S, 61°52′W, 15 Oct. 1950, *Burkart 18465* (SI) [LG, St]; Partido Tigre, Tigre, FNGBM, 34°25′S, 58°35′W, 13 Oct. 1946, *Lanfranchi 535* (SI) [LG, St]. **Entre Ríos:** Depto. Federación, Federación, 31°01′S, 57°53′W, 23 Sep. 1961, *Burkart 22437* (BAA) [LG, Pi and St]; Salto Grande, 31°13′S, 57°56′W, 10 Oct. 1950, *Hunziker 4431* (SI) [LG, Pi]; Depto. Uruguay, Concepción del Uruguay, 32°29′S, 58°14′W, 17 Oct. 1949, *Burkart 17969* (BAA) [LG, Pi and St]. PARAGUAY. **Alto Paraná:** Santa Teresa, 24°43′S, 54°21′W, 4 Aug. 1945, *Bertoni 1706* (BAA, SI) [LG, Pi]. URUGUAY. **Montevideo:** *Sellow s.n.* (isotype, BAA) [LG, Pi and St].

Poa lanuginosa

ARGENTINA. **Buenos Aires:** Partido Dorrego, Monte Hermoso, 38°59′S, 61°17′W, 30 Oct. 1965, *Cabrera et al. 17065* (LP) [LA, Pi and St]; Partido General Madariaga, Pinamar, 37°07′S, 56°51′W, 13 Oct. 1973, *Zardini 215* (LP) [LA, Pi]; Partido Junin, Médano Grande, 34°27′S, 61°04′W, 17 Oct. 1940, *Cabrera 6503* (LP) [LA, St]; Partido Tornquist, Sierra de la Ventana, estancia El Carol, 38°09′S, 61°55′W, 6 Oct. 1939, *Cabrera 5309* (LP) [LA, St]. **Chubut:** Depto. Río Sengüerr, Estancia La Laurita, 44°44′S, 70°15′W, 18 Jan. 1949, *Soriano 3849* (BAA) [PA, Pi]; Río Mayo, Estación Zootecnia Mallín, El Tacho, 45°41′S, 70°16′W, 4 Feb. 1954, *Grondona 3545* (BAA) [BO, Pi]; Depto. Florentino Ameghino, Cabo Raso, 44°20′S, 65°14′W, *Spegazzini 938* (holotype, BAA fragment) [BU, Pi], Jan. 1922, *Grether 3447* (BAA) [PA, PI]. **Mendoza:** Depto. San Rafael, Valle del Atuel, El Sosneado, 35°05′S, 69°34′W, 4 Oct. 1955, *Böcher et al. 801* (holotype, BAA) [BO, Pi and St]. **Neuquén:** Depto. Los Lagos, Nahuel Huapi, Estancia Fortín Chacabuco, 41°03′S, 71°09′W, 3 Nov. 1949, *Boelcke & Hunziker 3492* (BAA) [LA, Pi]; Depto. Minas, Confluencia ríos Pichi-Neuquén y Neuquén, Pampa de las Yeguas, 36°35′S, 70°48′W, *Boelcke et al. 13676* (BAA) [LA, Pi and St]; a 5 km de Las Ovejas, camino a la laguna Epu-Lauquén, 36°57′S, 70°45′W, 14 Jan. 1964, *Boelcke et al. 10772* (BAA) [LA, Pi]. **Río Negro:** Depto. Avellaneda, ruta 22, 50 km antes de Choel choel, 39°10′S, 65°05′W, *Bacigalupo N. & Nicora E. 11651 1/2* (BAA) [BO, Pi]; Depto. Norquinco, Río Chico, 41°42′S, 70°27′W, 9 Nov. 1949, *Soriano 3751* (BAA) [BO, Pi and St]; Depto. Avellaneda, ruta 250, 126 km antes de General Conesa, 39°45′S, 65°26′W, 7 Nov. 1972, *Bacigalupo & Nicora 11676* (BAA) [LA, Pi]; Isla Choel Choel, orilla del Río Negro, 39°16′S, 65°39′W, 7 Nov. 1972, *Bacigalupo & Nicora 11654* (BAA) [LA, Pi and St]; Choel choel, 39°10′S, 65°05′W, *Bacigalupo & Nicora 11651* (BAA) [BO, St]; Depto. Pichi Mahuida, a 20 km S de Río Colorado, 39°03′S, 64°20′W, 7 Nov. 1965, *Correa & Nicora 3168* (BAA) [LA, Pi and St]. **Santa Cruz:** Depto. Magallanes, San Julián, 5 km al sur por ruta 3, Bajo Salado, 49°19′S, 67°42′W, 22 Nov. 1963, *Correa et al. 2683* (BAB) [PA, Pi]; Depto. Corpen Aike, Río Chico, Establecimiento Las Vegas, 50°07′S, 68°37′W, Nov. 1922, *Dauber 165* (BAA) [PA, Pi and St]; Orilla del Río Chico, ruta 3, 49°49′S, 68°37′W, 3 Dec. 1971, *Boelcke et al. 15345* (BAB) [BU, Pi]; Depto. Deseado, Puerto Deseado, Cañadón del Veneciano, 47°45′S, 65°54′W, 12 Nov. 1965, *Correa & Nicora 3317* (BAA) [PA, St]; Depto. Lago Argentino, Campos de Bilbao, Monte Buenos Aires, 50°38′S, 72°54′W, 3 Feb. 1914, *Hauman et al. 152* (BAA) [PA, St]; Depto. Río Chico, Cañadón León, 48°46′S, 70°16′W, Mar. 1952, *Cittadini 18* (BAA) [PA, St], *Cittadini 21* (BAA) [BU, Pi]. **Tierra del Fuego e Islas del Atlántico Sur:** Depto. Río Grande Cabo Domingo, a 15 km NW de Río Grande, 53°41′S, 67°50′W, 3 Jan. 1968, *Moore 1493* (BAA) [PA, Pi]. CHILE. **XII Región:** Lago Pinto, cerca del origen del río, 52°00′S, 72°24′W, 11 Dec. 1877, *Ibar s.n.* (holotype, BAA fragment) [PA, St].

Poa ligularis

ARGENTINA. **Buenos Aires:** Partido Bahía Blanca, Bahía Blanca, 38°44′S, 62°16′W, *Hanslow 552* (type, BAA fragment) [LI, St], *Claraz 34* (*P. denudata* var *minor* Ball, BAA fragment) [LI, St]. **Chubut:** Depto. Biedma, Puerto Madryn, Laguna Blanca, 42°47′S, 65°02′W, 18 Oct. 1990, *Bertiller & Beeskow 1132* (SI) [LI, St], 30 Oct. 1992, *Bertiller 3150* (SI) [LI, Pi]; Estancia Sarasa a 40 km de Puerto Madryn, 42°37′S, 65°22′W, 11 Oct. 1995, *Bertiller 3395* (SI) [LI, St], *Bertiller 3411* (SI) [LI, Pi]; Depto. Rawson, Trelew, 43°15′S, 65°19′W, 16 Oct. 1946, *Soriano 1867* (SI) [LI, Pi]. **La Pampa:** Depto. Chical-Co, Cerro Los Guanacos, Oct. 1960, *Cano 1119* (BAB) [LI, St]; Depto. Utracán, Entre Atreucó y Quehué, 37°05′S, 64°11′W, 10 Jan. 1995, *Ragonese & Piccinini 18205* (BAB) [LI, Pi]. **Mendoza:** Depto. Las Heras, Puesto La Obligación, 20 Nov. 1943, *Covas 2097* (SI) [LI, Pi]. **Neuquén:** Depto. Confluencia, alrededores de la ciudad de Neuquén, 38°58′S, 68°03′W, Jan. 1978, *León 2232* (BAA) [LI, Pi], *León 2257* (BAA) [LI, Pi and St]. **Río Negro:** Depto. Avellaneda, ruta 22, 50 km antes de Choel Choel, 39°10′S, 65°05′W, 6 Nov. 1972, *Bacigalupo & Nicora 11646* (SI) [LI, Pi]; Depto. Conesa, ruta 251 a 13 km al N de General Conesa, camino a Río Colorado, 40°01′S, 64°27′W, *Bacigalupo & Nicora 12503* (BAA) [LI, Pi and St]; Depto. San Antonio, Sierra Grande, 41°38′S, 65°23′W, *Piccinini & Leguizamón 1610* (BAA) [LI, Pi]; ruta 3 a 8 km sur de San Antonio, 40°45′S, 64°56′W, *Correa & Nicora 3193* (BAA) [LI, Pi and St]. **Santa Cruz:** Depto. Deseado, Camino a Cabo Blanco, Tellier, entre Estancia El Chara y Tellier, 47°26′S, 66°02′W, *Correa et al. 3362* (BAA) [LI, Pi and St].

Poa pilcomayensis

ARGENTINA. **Entre Ríos:** Depto. Concordia, Parque Rivadavia, 31°23′S, 58°01′W, 22 Sep. 1961, *Burkart 22456* (SI) [PI, Pi and St]; Depto. Gualeguay, La Verde, 21 Nov. 1949, *Burkart 18064* (SI) [PI, Pi and St]; Islas Lechiguanas, Delta medio frente a Ramallo, 33°29′S, 60°01′W, 30 Dec. 1941, *Burkart 12874* (SI) [PI, St]; Depto. Victoria, Isla del Francés frente a Rosario, 32°57′S, 60°39′W, 15 Dec. 1937, *Burkart 8854* (SI) [PI, Pi and St], *Burkart 8817* (SI) [PI, Pi].

Poa pogonantha

ARGENTINA. **Patagonia:** Port Eden, 24 Jan. 1879, *Savatier 1844* (*Festuca pogonantha* Franch., holotype, BAA fragment) [PG, Pi]. **Chubut:** Depto. Futaleufú, Parque Nacional Los Alerces, Laguna Cisne, 42°36′S, 71°51′W, 16 Dec. 1962, *Roquero 5389* (BAA) [PG, Pi]; Depto. Río Sengüerr, Lago Fontana, Estancia La Pepita, 44°55′S, 70°58′W, 29 Jan. 1960, *Soriano 5662* (BAA) [PR, Pi]; Lago Fontana, Lote 15, 44°56′S, 71°30′W, *Martinoli & Boggiano 15080* (BAA) [PR, Pi and St], *Martinoli & Boggiano 15079* (BAA) [PR, St], 11 Feb. 1932, *Castellanos 9968* (BAA) [PR, Pi], *Martinoli & Boggiano 15077* (BAA) [PR, St]; *Martinoli et Boggiano 15078* (BAA) [PR, St]; Lago Fontana, Estancia La Pepita, 44°55′S, 70°58′W, 29 Jan. 1960, *Soriano 2618* (BAA) [PR, Pi]; Depto. Tehuelches, Lago Vintter, 43°58′S, 71°33′W, 2 Feb. 1989, *Nicora 9473 b* (SI) [PG, St]; Lago Vintter, playa arenosa, 43°58′S, 71°33′W, 7 Feb. 1989, *Nicora 9537* (SI) [PG, Pi]. **Río Negro:** Depto. Bariloche, Parque Nacional Nahuel Huapi, Ventisquero Frías, 41°11′S, 71°49′W, 13 Jan. 1952, *Boelcke & Correa 5500* (BAB) [PG, Pi and St]; Laguna Frías, 41°04′S, 71°49′W, 8 Jan. 1952, *Boelcke & Correa 5373* (BAB) [PG, Pi], *Boelcke & Correa 5380* (BAA) [PG, Pi]; Lago Roca, Arroyo Apoco, 41°23′S, 71°47′W, 26 Jan. 1952, *Boelcke & Correa 6045 1/2* (BAA) [PG, St]. **Tierra del Fuego e Islas del Atlántico Sur:** Depto. Ushuaia, Glaciar Martiales, 54°45′S, 68°18′W, 9 Dec. 1962, *Correa et al. 5365* (BAA) [PG, Pi]. CHILE. **XII Región:** Lago Azul, 51°27′S,

73°18′W, 10 Jan. 1977, *Moore & Pisano 1575* (BAB) [PG, Pi]; Islas Wollaston, Caleta Lientur, 55°44′S, 67°19′W, 23 Jan. 1980, *Pisano 5112* (SI) [PG, St]; Estancia Cerro Castillo, Cerro Solitario, 51°20′S, 72°37′W, 18 Jan. 1977, *Latour et al. 1739* (BAB) [PG, Pi]; Península W desembocadura Río Serrano, 51°25′S, 73°04′W, 23 Jan. 1977, *Moore & Pisano 1905* (BAB) [PG, Pi], *Moore 367* (SI) [PG, Pi].

Poa resinulosa complex

Poa buchtienii: BOLIVIA. **La Paz:** Manco Kapac, Lago Titicaca, Isla del Sol, 16°03′S, 69°10′W, 26 Jan. 1986, *Liberman 1289* (LPB) [BT, St]; Murillo, La Paz-Calacoto, 64 km hacia el nevado Illimani, 16°40′S, 67°45′W, 19 Jan. 1983, *Beck 9076* (LPB) [BT, Pi]. **Oruro:** Avaroa entre Challapata y Tolapalca, 19°20′S, 66°50′W, Feb. 1979, *Ceballos et al. 236* (SI) [BT, Pi]; La Paz, 18°36′S, 66°55′W, Feb. 1911, *Buchtien O. 2467* (syntype, BAA) [BT, St]; Poopo, a 4.5 km al norte de La Paz, sobre la ruta hacia Oruro, 16°20′S, 68°05′W, 6 Mar. 1993, *Peterson et al. 12714* (LPB) [BT, Pi and St]. **Potosí:** Surchichas, Potosí, a 12 km al NW de Salo, 20°55′S, 66°18′W, 21 Mar. 1992, *Peterson & Annable 11823* (LPB) [BT, Pi and St]. **Tarija:** Avilez Cerca de Cobres, 21°38′S, 64°47′W, 29 Jan. 1986, *Bastión 614* (LPB) [BT, Pi and St]. *P. calchaquiensis*: ARGENTINA. **Jujuy:** Depto. DR. Manuel Belgrano, Camino de huella de Alto Lozano a Tiraxi, 24°05′S, 65°40′W, 3 Nov. 1974, *Correa et al. 6106* (SI) [CL, St]; Depto. Humahuaca, Mina Aguilar, 23°12′S, 65°41′W, 23 Feb. 1972, *Ruthsatz 14577* (BAA) [CL, Pi]; Abra entre Iruya e Iturbe, 22°58′S, 65°21′W, 25 Jan. 1972, *Ruthsatz 14589* (BAA) [CL, St]; Depto. Santa Bárbara, El Fuerte, 22°07′S, 65°26′W, 18 Feb. 1972, *Cabrera et al. 22236* (BAA) [CL, Pi]; Depto. Susques, Cerro Tuzgle, 23°26′S, 66°30′W, 3 Mar. 1967, *Werner 76* (LP) [CL, Pi]; Depto. Tumbaya, Volcán, Abra del Paraguay, 23°55′S, 65°27′W, Feb. 1927, *Venturi 4905* (SI) [CL, St]; Depto. Yavi, Yavi Chico, 22°06′S, 65°28′W, 7 Mar. 1940, *Meyer 14926* (BAA) [CL, St]; Quebrada de Roquero, 22°18′S, 65°35′W, 20 Feb. 1963, *Cabrera 15369* (SI) [CL, Pi]. **Salta:** Depto. Guachipas, Pampa Grande, 25°52′S, 65°30′W, Mar. 1900, *Holmberg 2616* (BAA) [CL, Pi]. **Tucumán:** Depto. Taff, Cumbres Calchaquíes, 26°35′S, 65°40′W, 29 Jan. 1907, *Lillo 5065* (isotype, BAA) [CL, St]. *P. pedersenii*: PARAGUAY. **Caaguazú:** Yhu, 25°01′S, 55°58′W, 19 Sep. 1988, *Pedersen 15049* (holotype, SI) [PE, Pi and St]. *P. resinulosa*: ARGENTINA. **Buenos Aires:** Partido General Pueyrredón, Estancia La Brava, Sierra de Valdez, 37°54′S, 58°00′W, 18 Nov. 1977, *Boelcke et al. 791* (SI) [RE, Pi]; Partido Tornquist, Estancia Chica y Estancia Fortín Chaco, Cerro La Vieja, 38°06′S, 62°14′W, 17 Nov. 1981, *Villamil 2073* (SI) [RE, Pi and St]; Estancia Mamim-Co, Cerro del Potrero, 38°03′S, 61°56′W, 18 Nov. 1981, *Villamil 2110* (SI) [RE, St], *Villamil 2054* (SI) [RE, Pi and St]. **Chubut:** Depto. Futaleufú, Estancia Suñica, 43°03′S, 71°04′W, *Lahitte s.n.* (BAA) [RE, Pi]; Parque Nacional Los Alerces, Río Percey, Cerro Ceballos, 42°55′S, 71°20′W, *Lahitte & Roquero 192* (BAA) [RE, St]; Depto. Languiñeo, Río Tecka, Pampa Chica, 43°28′S, 70°51′W, 8 Nov., *Skottsberg s.n.* (*P. decolorata* Pilg., isotype, BAA fragment) [RE, St]. **Córdoba:** Depto. Río Seco, Caña Cruz a 10 km de Villa de María, 29°54′S, 63°44′W, 7 Nov. 1949, *Hunziker 8003* (SI) [RE, St]. **Mendoza:** Mendoza, *Gillies s.n.* (isotype, BAA, fragment) [RE, Pi]. **Neuquén:** Depto. Huiliches, Parque Nacional Lanin, a Lago Tromen, 39°34′S, 71°32′W, *Lahitte et al. 606* (BAA) [RE, Pi and St]; San Martín de los Andes, camino entre Lolog y Mamuil-Malal, 39°40′S, 71°22′W, *Neumeyer 31* (BAA) [RE, Pi and St]; Depto. Los Lagos, Estancia Fortín Chacabuco, 41°02′S, 71°15′W, *Boelcke 3210 1/2* (BAA) [RE, Pi and St]. **Santa Fe:** Depto. Vera, Calchaquí, 29°54′S, 60°18′W, 7 June 1965, *Alonso 866* (SI) [RE, Pi].

Poa rigidifolia

ARGENTINA. **Santa Cruz:** Depto. Güer Aike, Estancia Stag River, meseta Latorre, Cerro Punta Gruesa, 51°32′S, 72°05′W, 25 Jan. 1978, *Roig et al. 2950* (BAB) [RI, Pi]; Estancia Punta Alta, 51°43′S, 71°58′W, 24 Jan. 1976, *Latour et al. 536* (BAB) [RI, Pi]; Estancia Punta Loyola, 51°44′S, 68°56′W, 1976, *Nicora 828* (BAB) [RI, Pi]; 42 km oeste de Estancia Punta del Monte, cruce a Sección Magán, 51°32′S, 71°35′W, 1977, *Roig & Méndez 2494* (BAB) [RI, Pi and St]; entre Estancia Glencross y Laguna Cóndor, 51°48′S, 71°42′W, 14 Dec. 1976, *Latour et al. 1057* (BAB) [RI, St]; Estancia Primavera, ruta 293, 51°27′S, 72°15′W, 1976, *Latour et al. 1198* (BAB) [RI, Pi]; Punta Loyola, 51°50′S, 69°11′W, 6 Dec. 1976, *Latour et al. 906* (BAB) [RI, St]; Bajo La Leona, 51°31′S, 69°46′W, 11 Nov. 1977, *Roig & Méndez 2437* (BAB) [RI, St]; Río Gallegos, entre Estancia Maragata y Las Buitreras, 51°45′S, 70°10′W, 10 Dec. 1975, *Arroyo et al. 410* (BAB) [RI, St]; Estancia Guakenken Aike, 51°27′S, 69°48′W, 14 Nov. 1977, *Roig & Méndez 2485* (BAB) [RI, Pi and St]. **Tierra del Fuego e Islas del Atlántico Sur:** Depto. Río Grande, Estancia El Salvador, 53°39′S, 68°35′W, 20 Nov. 1971, *Boelcke et al. 15093* (BAB) [RI, Pi]; 51 km N de San Sebastián, Estancia Cullen, 52°53′S, 68°30′W, 21 Nov. 1971, *Boelcke et al. 15156* (BAA) [RI, Pi]; Estancia Secunda Argentina, Jan. 1933, *Castellanos 7597* (BAA) [RI, Pi]; Islas Malvinas, Port William, 52°00′S, 59°00′W, Sep. 1850, *Lechler s.n.* (*P. rigidifolia* Steud., holotype, BAA fragment) [RI, Pi]. CHILE. **XII Región:** Aestate, Mar. 1936, *Philippi s.n.* (*P. poecila* Phil., isotype, BAA fragment) [RI, Pi]; Sandy Point, 53°10′S, 70°54′W, Oct. 1852, *Lechler 1068b* (*Aira spiciformis* Steud., holotype, BAA fragment) [RI, Pi].

Poa rigidifolia var. *ibari*

ARGENTINA. **Chubut:** Depto. Ameghino, Lochiel, 44°39′S, 66°10′W, 19 Oct. 1946, *Soriano 1915* (BAA) [DU, Pi and St]. **Santa Cruz:** Depto. Güer Aike, Estancia Guakenken Aike, 51°25′S, 69°48′W, *Roig et al. 2488* (BAB) [DU, Pi]; ruta 3, cruce con camino a Puerto Coyle, 51°05′S, 69°27′W, 24 Nov. 1963, *Correa et al. 2790* (BAA) [IB, Pi]; Estancia La Carlota sección San Elías, 51°24′S, 71°24′W, 20 Jan. 1978, *Roig et al. 2865* (BAA) [IB, St]; Río Chico, Chimen Aike, 51°44′S, 69°18′W, 26 Nov. 1950, *Sleumer 790* (BAA) [IB, Pi]; Cumbre de la cordillera Chica, Cerro Punta Alta, 51°27′S, 72°06′W, 5 Feb. 1978, *Ambrosetti & Méndez 3747* (BAA) [IB, Pi]; Sección San Antonio, 51°24′S, 71°34′W, 19 Jan. 1978, *Roig et al. 2756* (BAA) [IB, Pi and St]; Estancia Cóndor, 51°50′S, 69°11′W, 8 Dec. 1976, *Latour et al. 877* (BAB) [IB, St]; Bajo La Leona, 51°31′S, 69°46′W, 1977, *Roig & Méndez 2436* (BAB) [IB, St]; Estancia Los Vascos, 51°25′S, 70°52′W, 21 Jan. 1978, *Roig et al. 2877* (BAB) [IB, St]; Depto. Lago Argentino, ruta 40, a 100 km al NW de La Esperanza hacia Lago Argentino, 50°21′S, 71°40′W, 28 Nov. 1963, *Correa et al. 2888* (BAA) [DU, Pi and St]; Depto. Deseado, Patagonia orientalis ad Mazaredo portum, 47°05′S, 66°42′W, Jan. 1905, *Dusén 5318* (*P. dusenii* Hack., holotype, BAA fragment) [DU, Pi]; Camino a

Puerto Deseado, 47°48′S, 66°12′W, 18 Nov. 1963, *Correa et al. 2590* (BAA) [DU, Pi and St]; Depto. Río Chico, Camino a Gobernador Gregores, Hotel Las Horquetas, 48°13′S, 71°19′W, *Correa & Nicora 3511* (BAA) [DU, Pi and St]. CHILE. **XII Región:** Lago Pinto, 52°00′S, 72°24′W, Jan. 1844, *Ibar s.n.* (*P. ibari* Phil., isotype, BAA fragment) [IB, Pi].

Poa schizantha

ARGENTINA. **Buenos Aires:** Partido Bahía Blanca, Bahía Blanca, 38°44′S, 62°16′W, Nov. 1941, *Zaffanella 268* (BAB) [SC, Pi]; Partido Dorrego, Monte Hermoso, en las dunas marítimas, 38°59′S, 61°17′W, 8 Nov. 1940, *Parodi 13672* (holotype, BAA, SI) [SC, Pi], *Parodi 13673* (BAA) [SC, St], *Parodi 13675* (BAB) [SC, Pi], *Cabrera 6752* (BAA) [SC, Pi], Jan. 1941, *Zaffanella 14402* (BAA) [Sc, Pi].

Poa tristigmatica

ARGENTINA. **Chubut:** Departamento Río Sengüerr, Pampa de Chalía, Estancia La Media Luna, 45°35′S, 71°27′W, 3 Dec. 1981, *Villamil 2236* (SI) [TR, St]; Lago La Plata, 44°51′S, 71°53′W, *Martinoli & Boggiano 15073* (BAA) [TR, Pi]; Depto. Tehuelches, Gobernador Costa a Río Pico, 44°07′S, 70°55′W, 14 Dec. 1972, *Latour et al. 3588* (SI) [TR, Pi]. **Neuquén:** Depto. Lácar, Cerro Chapelco, en el filo, 40°09′S, 71°20′W, 23 Feb. 1974, *Correa et al. 5926* (*P. boelckei* Nicora, holotype, BAB) [BK, Pi], *Correa et al. 5928* (BAB) [BK, Pi]; Cordón del Cerro Chapelco, Portezuelo Trahunco, Faldeo S, 40°09′S, 71°20′W, 13 Feb. 1978, *Gentili 695* (BAB) [BK, St]; Cerro Chapelco, 40°09′S, 71°20′W, Feb. 1961, *León & Calderón 963* (BAA) [BK, Pi], 26 Jan. 1966, *Eskuche 603-2* (BAA) [BK, St], *Eskuche 603-1* (BAA) [BK, St], *Eskuche 599-3* (BAA) [BK, Pi], 12 Jan. 1961, *León & Calderón 903* (BAA) [BK, Pi]; Depto. Minas, Paso del Macho, 36°26′S, 70°46′W, 26 Jan. 1970, *Boelcke et al. 13931* (BAA) [TR, Pi], *Boelcke et al. 13926* (BAA) [TR, St]; Laguna Varvarco Campos, Cajón Benítez, paso Puerta Vieja, 36°17′S, 70°41′W, 1 Nov. 1970, *Boelcke et al. 14281* (BAA) [TR, Pi]; Cordillera del Viento, cruzada de Tricao Malal al Cajón de Butaló, 36°58′S, 70°30′W, 3 Nov. 1964, *Boelcke et al. 11568* (BAB) [TR, St]; Cajón del Portillo, 2560 m, 36°12′S, 70°36′W, 31 Jan. 1970, *Boelcke et al. 14168* (BAA) [TR, Pi and St]; Cordillera del Viento cruzada de Tricao Malal al Cajón de Butaló, en vertiente, 36°58′S, 70°30′W, 3 Nov. 1964, *Boelcke et al. 11567* (BAA) [TR, St]; Depto. Ñorquín, Copahue, 37°49′S, 71°06′W, 3 Feb. 1930, *Hirschhorn 23* (BAA) [TR, Pi and St]. **Río Negro:** Depto. Nahuel Huapi, Cerro Catedral, 41°05′S, 71°45′W, Feb. 1954, *Parodi 15343* (BAA) [TR, Pi], *Parodi 15323* (BAB) [TR, Pi]. CHILE. **XII Región:** Cordillera de Talcaregue, Feb. 1831, *Gay 49* (*P. tristigmatica* E. Desv., in Gay, syntype, BAA fragment) [TR, Pi].

Appendix 3.

Morphological variables used in numerical analyses, their abbreviations in parentheses, codification, and brief explanations of character variation.

Vegetative Variables

1. Plant habit (HAB). Caespitose or short-rhizomatous (1), long-rhizomatous (2), stoloniferous (3). Plants range from typically caespitose like *P. ligularis* to long-rhizomatous as *P. lanuginosa*. Caespitose plants sometimes develop stolons as in *P. alopecurus* or long rhizomes as in *P. tristigmatica*.
2. Leaf length (LEAle, cm). This is a variable character within *Poa* sect. *Dioicopoa*, although there is a conspicuous discontinuity between large-sized species and smaller ones. This character usually correlates with sheath and leaf blade length, plant height, panicle length, and number of nodes on principal panicle axis. The highest value, 93 cm, was measured on *P. dolichophylla*; the minimum value, 3.5 cm, corresponded to *P. rigidifolia*.
3. Sheath length (SHEle, cm). The highest value found, 39 cm, corresponded to *P. bergii* and *P. stuckertii*, while the minimum value, 1.7 cm, was measured on *P. rigidifolia*.
4. Leaf blade length (BLAle, cm). The highest value found, 61 cm, corresponded to *P. dolichophylla*, while the minimum value, 1.3 cm, was measured on *P. rigidifolia*.
5. Ligule length (LIGle, mm). This character presents a conspicuous discontinuity, dividing *Poa* sect. *Dioicopoa* into short-liguled species and long-liguled ones. The longest ligule, 19.6 mm, was measured in *P. bergii*; the shortest, 0.20 mm, in *P. pedersenii*.
6. Ligule shape (LIGsp). Acuminate (1); truncate to obtuse (2). Long ligules are usually acuminate (*P. ligularis*), while shorter ligules are commonly truncate (*P. bonariensis*). Species like *P. denudata* can have acuminate ligules of short to medium lengths.
7. Leaf blade apex (API). Navicular or obtuse (1); sharp or acuminate (2). *Poa* sect. *Dioicopoa* is characterized by a navicular or obtuse leaf blade apex as in *P. lanigera*. Sharp or acuminate apices also occur within species such as *P. hubbariana* and *P. bergii*.

Blade Cross Sections

8. Blade outline (OUTli). Flat (1); conduplicate (2); convolute or subconvolute (3). Species of the *P. dolichophylla* complex are characterized by flat blades. However, *P. schizantha* presents a convolute to subconvolute blade, probably associated with the absence of bulliform cells; blades in other species vary from conduplicate to subconvolute.
9. Blade width, measured on the adaxial epidermis between blade margin and midrib (BLAw, mm). This measure generally correlates with the number of vascular bundles with sclerenchyma girders on both adaxial and abaxial epidermis. The highest value was recorded in *P. iridifolia*, 6.8 mm, and the minimum value was found in *P. ligularis*, 0.37 mm.
10. Blade maximal thickness between abaxial and adaxial epidermis layers (BLAt, mm). This character frequently correlates with stomata length. The maximum value, 0.55 mm, was seen in *P. bergii*, and the minimum value, 0.10 mm, in *P. pilcomayensis*.
11. Bulliform cell development (BULc). Not to little differentiated (1); inflated, and well developed (2). *Poa* sect. *Dioicopoa* presents bulliform cell groups at both sides of the midrib as in *P. holciformis*. *Poa schizantha* is the only species that lacks bulliform cells, while these are generally little differentiated in *P. pogonantha*.
12. Shape of sclerenchyma at blade margin (CAP). Rounded to pointed cap, without sclerenchyma extending on abaxial or adaxial epidermis (1); crescent-shaped cap, with sclerenchyma briefly extending on

abaxial and adaxial epidermis (2) (terminology of states as in Ellis, 1976). *Poa* sect. *Dioicopoa* generally presents a group of sclerenchyma cells at blade margin, seen in cross section. Terminal sclerenchymatous caps are commonly rounded to pointed like in *P. rigidifolia*, with species of the *P. dolichophylla* complex presenting a crescent-shaped cap.

13. Number of vascular bundles with sclerenchyma girders on both adaxial and abaxial epidermis (SCL2). Highest values were recorded in species of the *P. dolichophylla* complex (45), whereas minimum values of only 1 or 2 were found in *P. rigidifolia*, *P. ligularis*, and the *P. resinulosa* complex, among others.
14. Number of vascular bundles with a sclerenchyma girder only on abaxial epidermis (SCLab). These are commonly few (1 or 2) in the *P. dolichophylla* complex, but are more numerous (5 to 8) in *P. bergii*.
15. Number of vascular bundles with a sclerenchyma girder only on adaxial epidermis (SCLad). Rare in *Poa* sect. *Dioicopoa*, they are often present (3 to 5) in the *P. dolichophylla* complex.
16. Number of vascular bundles with only a few sclerenchyma cells surrounding the bundles (SCLin). This character is variable in species of *Poa* sect. *Dioicopoa*, but generally few vascular bundles correspond to this type.

ABAXIAL BLADE EPIDERMIS

17. Stomata length (STOM, mm). This character, usually indicative for different ploidy levels, presents three ranges of variation. Large stomata are seen in *P. alopecurus*, *P. bergii*, *P. bonariensis*, *P. holciformis*, *P. indigesta*, *P. lanuginosa*, *P. pogonantha*, and *P. tristigmatica*. Small stomata are seen in the *P. dolichophylla* complex, whereas other species have intermediate values.
18. Thickness of the long cells (CELt, mm). This varies between 0.016 mm in *P. ligularis* and 0.033 mm in *P. lanuginosa*.
19. Abaxial epidermal prickles (PRIC). Absent to infrequent (1); frequent to numerous (2). Prickles are generally frequently distributed on abaxial epidermis, although they are infrequent in the *P. dolichophylla* complex, *P. bergii*, *P. hubbardiana*, *P. lanigera*, *P. pilcomayensis*, and *P. tristigmatica*.
20. Silico-suberose cell pairs on intercostal epidermis (SISU). Absent to infrequent (1); frequent to numerous (2). Silico-suberose paired cells are the usual condition in *Poa* sect. *Dioicopoa*, though they are infrequent in *P. lanigera* and *P. pilcomayensis*.

FERTILE VARIABLES

21. Plant height, measured from the longest culm of fertile plants (HEIG, cm). Culms usually exceed leaves, except in *P. hubbardiana*, where they are as long as leaves, occasionally longer. The tallest plant was recorded in the *P. dolichophylla* complex (136 cm); the smallest plant, in *P. rigidifolia* (6 cm).
22. Number of culm nodes (CULM). This varies between 2 and 3 in small plants and 4 to 5 in taller plants.
23. Panicle length (PANle, cm). The longest panicle (37 cm) was recorded in *P. dolichophylla*; the shortest (1.7 cm) was in *P. rigidifolia*.
24. Panicle width, measured on widest panicle (PANw, cm). Panicles are contracted within *Poa* sect. *Dioicopoa*, with the widest recorded in *P. bonariensis* (2–5 cm). The *Poa dolichophylla* complex presents broad panicles (5–8 cm), which are usually expanded.
25. Number of nodes on principal panicle axis (PANn). This varies from 20 in the *P. dolichophylla* complex, *P. bergii*, *P. bonariensis*, *P. ligularis*, and *P. indigesta*, to only 6 in *P. alopecurus*, *P. pogonantha*, and *P. rigidifolia*.
26. Length of terminal, well-developed spikelet of a branch panicle (SPIle, mm). Well-developed spikelets were selected from the upper half of a panicle, particularly from terminal spikelets of a branch. Spikelet length and correlated measures do not usually vary within the same panicle. The highest value was found in *P. pogonantha* (28 mm), although it is associated with vivipary. The longest non-viviparous spikelets were found in *P. bergii* and *P. schizantha* (12 mm), with the shortest among species of the *P. dolichophylla* complex (2.8 mm).
27. Width of terminal well-developed spikelet of a branch panicle (SPIw, mm). This character correlates with flower number per spikelet. The broadest spikelet was recorded in *P. bergii* (10 mm).
28. Number of florets per spikelet (FLOn°). Spikelets in *Poa* sect. *Dioicopoa* generally have 3 to 5 flowers. Fifteen flowers were found in *P. schizantha*, and 11 in *P. bergii*. The minimum value observed, (2), is commonly present among species of *Poa*.
29. Viviparous florets (FLOv). Absent (0); present (1). Vivipary is a reproductive alternative found in species inhabiting low-temperature areas with a short fertile season, such as *P. alopecurus*, *P. pogonantha*, and *P. tristigmatica*.
30. First glume length (GLUle, mm). This correlates with lemma and palea length as well as glume, lemma, and palea width, and is less correlated with lodicule length and width. This character set varies among pistillate and staminate plants. The longest glumes were observed on pistillate specimens of *P. bergii* (10.3 mm) and *P. alopecurus* (8.9 mm). The shortest glumes were recorded in a staminate specimen of *P. dolichophylla* (1.38 mm).
31. First glume width, measured from principal nerve to margin (GLUw, mm). This varies from 0.33 mm in *P. dolichophylla* and *P. bonariensis* to 1.45 mm in *P. alopecurus*, being as much as 1.68 mm in *P. bergii*.
32. Ratio of first glume length/second glume length (G1/G2). Equal to 1 (0); less than 1 (1); more than 1 (2). Having the first glume shorter than the second glume is typical in *Poa* sect. *Dioicopoa*. However, *P. stuckertii* commonly presents glumes of equal lengths.
33. Ratio of second glume length/lemma length (G2/LE). Equal to 1 (0); less than 1 (1); more than 1 (2). The second glume is generally shorter than the lower lemma in *Poa* sect. *Dioicopoa*.
34. Number of nerves on the first glume (GLUn). Glumes in *Poa* sect. *Dioicopoa* generally present a principal nerve and two marginal nerves (sometimes absent). *Poa bergii* features two additional marginal nerves (in total, 3 to 5, up to 7).
35. Prickles on the rachilla (RACH). Absent to infrequent (0); frequent to numerous prickles (1). *Poa* sect. *Dioicopoa* generally present these prickles on rachilla and panicle axes.
36. Lemma length (LEMle, mm). Dimorphism between pistillate and staminate plants impacts lemma lengths within species of *Poa* sect. *Dioicopoa*. Species discrimination is considered among specimens of the

same sex. The shortest lemma was recorded on a staminate specimen of *P. stuckertii* (2.58 mm); the longest on a pistillate specimen of *P. bergii* (10.33 mm).

37. Lemma width, measured from principal nerve to margin (LEMw, mm). Dimorphism between pistillate and staminate plants is not always evident in lemma width. The maximum value was recorded in *P. lanuginosa* (2.2 mm); the minimum value in species of the *P. resinulosa* complex (0.54 mm).
38. Palea length (PALle, mm). The shortest palea was recorded on a staminate specimen of the *P. resinulosa* complex (1.75 mm); the longest on a staminate specimen of *P. alopecurus* (6.72 mm).
39. Palea width between nerves (PALw, mm). Dimorphism between pistillate and staminate plants is not always evident in palea width. The maximum value was recorded in *P. bergii* (1.8 mm); the minimum value corresponds to species of *P. pilcomayensis* (0.30 mm).
40. Lodicule length (LODle, mm). Only entire and well-developed lodicules were considered for recording data. The longest lodicules were measured on *P. alopecurus, P. bergii, P. indigesta, P. lanuginosa, P. pogonantha, P. rigidifolia,* and *P. tristigmatica* (1.2–1.7 mm long). The shortest lodicules were recorded on *P. bonariensis* and *P. ligularis* (0.25–0.33 mm long).
41. Lodicule width, including lobes (LODw, mm). Species variation within *Poa* sect. *Dioicopoa* ranges between 0.2 mm and 0.7 mm wide, being more than 1 mm only in a few specimens of *P. huecu* and *P. tristigmatica.*
42. Hairs on the callus of the first floret (HAIcal). Absent (0); rigid and short, less than 1/2 of the floret (1); rigid and long, more than 1/2 of the floret (2); woolly and short, less than 1/2 of the floret (3); woolly and long, more than 1/2 of the floret (4). Hairiness is a dimorphic character between pistillate and staminate florets. Pistillate florets usually have a hairy callus in *Poa* sect. *Dioicopoa,* and pistillate florets of only *P. indigesta, P. huecu,* and *P. holciformis* are completely glabrous. Staminate specimens are usually glabrous, but they can also present a few rigid or woolly, long or short hairs.
43. Hairiness along lemma nerves (HAInrv). Absent (0); scabrous (1); hairs less than 0.5 mm (2); hairs more than 0.5 mm (3). Pistillate florets of *Poa* sect. *Dioicopoa* are characterized by the presence of hairs on principal and marginal nerves that are 0.5 mm long or less. *Poa bonariensis, P. lanigera, P. ligularis, P. lanuginosa,* and *P. hubbardiana* present hairs longer than 0.5 mm, these being conspicuous and abundant in *P. hubbardiana.* Staminate floret nerves are typically glabrous.
44. Pubescence between principal and secondary lemma nerves (HAIbet). Absent (0); scabrous (1); hairs less than 0.5 mm (2); hairs more than 0.5 mm (3). They are common on pistillate florets of *P. ligularis* and *P. rigidifolia,* being less than 0.5 mm long, as well as abundant and longer in *P. hubbardiana.*

STUDIES IN ANNONACEAE XXXVI. THE *DUGUETIA* ALLIANCE: WHERE THE WAYS PART[1]

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ABSTRACT

Results of a cladistic analysis of morphological and anatomical data of the *Duguetia* alliance (Annonaceae) are presented. Several novel characters, or characters so far not incorporated into phylogenetic analyses, are used. Parsimony analysis resulted in two strongly supported clades: a *Fusaea* clade, comprising *Fusaea, Duckeanthus, Letestudoxa,* and *Pseudartabotrys,* and a *Duguetia* clade, comprising all Neotropical and African species of *Duguetia.* Support for the past distinction of *Pachypodanthium* from *Duguetia* appears to be absent. Characters used to resolve relationships between and within the two clades are difficult to polarize by outgroup comparison. It is demonstrated that the critical reassessment of classical morphological characters, and the search for new ones, may well advance phylogenetic resolution within Annonaceae.

Key words: Annonaceae, cladistics, *Duckeanthus, Duguetia, Fusaea, Letestudoxa,* morphology, *Pachypodanthium, Pseudartabotrys.*

The classification of the Annonaceae presents workers on this family with a Herculean challenge. Early classifications of the family, as in Hooker and Thomson (1855), emphasize identification and only incidentally reflect phylogeny. This also applies to a more recent classification (Hutchinson, 1964). However, at higher, tribal levels, these two classifications have little in common. To date, the classification by Fries (1959) resolves most subgroups. Exclusively on the basis of inflorescence and floral characters, he distinguished two subfamilies, three tribes, and 14 informal genus groupings. The composition of many of these genus groups has been amended after phenetic analyses of flower and fruit morphology (van Heusden, 1992; van Setten & Koek-Noorman, 1992; Koek-Noorman et al., 1997), and phylogenetic analyses based on gross morphological and palynological data (Doyle & Le Thomas, 1994, 1996, 1997).

One of Fries's genus groups is the *Duguetia* alliance, comprising West African and tropical American genera. The composition of this alliance remained untouched to date except for the exclusion of *Malmea* and the inclusion of the monotypic genus *Pseudartabotrys* (van Setten & Koek-Noorman, 1992). Characteristic features of the genera belonging to this alliance include valvate sepals, imbricate petals, one basal ovule, and the presence of a rudimentary aril. Most distinctive for the alliance is the presence of pseudosyncarpous fruits. These are aggregates of astipitate carpels, which become fused with one another and/or adnate to the receptacle. Genera usually considered to fit into the *Duguetia* alliance are the Neotropical genera *Duckeanthus* (1 sp.), *Duguetia* (95 spp.), and *Fusaea* (2 spp.), together with *Letestudoxa* (3 spp.), *Pachypodanthium* (4 spp.), and *Pseudartabotrys* (1 sp.) from West Africa (van Setten & Koek-Noorman, 1992; Le Thomas et al., 1994; Koek-Noorman et al., 1997). Van Heusden (1992) dissentingly placed *Duguetia* and *Pachypodanthium* in one informal group, separate from *Duckeanthus, Fusaea, Letestudoxa,* and *Pseudartabotrys* (plus *Afroguatteria, Enicosanthellum,* and *Disepalum*) in another.

Thus, the majority opinion on the circumscription of the *Duguetia* alliance seems to prevail. Yet closer examination reveals some problems. Recent cladistic analyses (Doyle & Le Thomas, 1994, 1995, 1996, 1997; Doyle et al., 2000) array *Duguetia, Pachypodanthium, Letestudoxa, Fusaea,*

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and *Duckeanthus* in one clade, linked to the clade of the "xylopioids." *Pseudartabotrys* was not included in these analyses.

Pseudosyncarpy, which otherwise only occurs in the *Annona* group, is an obvious synapomorphy for this "*Duguetia* clade." During pseudosyncarpous fruit development the postgenital aggregating of the carpels can occur through two processes, viz. the lateral fusion of carpel walls, and the inclusion of the very basal parts of the carpels into the fruiting receptacle. The former case has been extensively documented for the genera *Annona* and *Rollinia* by Briechle-Mäck (1994). The fusion of carpels starts with dovetailing of the epidermal cells of adjacent carpels, and ends with complete fusion. The fruiting receptacle does not contribute to the aggregating of the fruit. A similar fruit development has been described for *Fusaea* (Chatrou & He, 1999). The inclusion of the very basal parts of the carpels into the fruiting receptacle (see Svoma, 1998) originates from acropetal development of the receptacle after flowering has been completed. This type of aggregating of the fruit is present in all species of *Duguetia*, whereas the degree of lateral fusion of the carpels varies from completely free to completely fused among the species of this genus. Thus, both origins of pseudosyncarpy occur within the *Duguetia* alliance and can be traced when closely inspecting fruit morphology. Pseudosyncarpy should therefore be considered as a non-homologous similarity. However, the fruit type can be incorporated into analyses in a more straightforward way by unravelling it ontogenetically (Patterson, 1982). In this analysis we will consider the differential origins of pseudosyncarpy, separating it into two morphological characters (see Data and Analyses).

A family-wide phenetic analysis based on flower and fruit morphology resulted in inclusion of *Duckeanthus, Letestudoxa, Pseudartabotrys, Fusaea,* and *Pachypodanthium* in one cluster (Koek-Noorman et al., 1997). *Duguetia* appeared in another cluster, together with *Guatteria* and the *Annona* group. Nevertheless, the overall similarity between all genera, including *Duguetia,* was perceived so strongly by the authors that the signal appearing from the phenogram was ignored, and all genera were grouped together in a tentative scheme of genus groups. In the same paper, Koek-Noorman et al. (1997) concluded that some of the principal characters used by Fries (1959) for the distinction of genus groups, viz. sepal and petal aestivation, barely contribute to their phenetic clustering.

The following paradox thus emerges: a genus group, or clade, has long been recognized intuitively but is weakly supported by morphological evidence. Schatz and Le Thomas (1993) stated that confusing phylogenetic patterns based on macromorphological character distribution within Annonaceae have been clarified during the past two decades by new palynological and karyological evidence. In spite of its general validity, this statement cannot be applied to the *Duguetia* alliance. Karyological evidence is too scattered to be unequivocal (Doyle & Le Thomas, 1996). Palynological data reveal too many autapomorphies among the genera of this alliance to be illuminating. Based wholly on palynological data, Walker (1971) even erected the informal *Fusaea* subfamily, accommodating *Fusaea* and *Duckeanthus,* but placed *Duguetia* in another subfamily. Walker's data were reinterpreted by Le Thomas (1980–1981) and Le Thomas et al. (1994). However, Le Thomas et al. (1994) did not clarify the phylogeny of the *Duguetia* alliance with pollen ultrastructural data, but conversely discussed the implications of their resulting phylogenies for the evolution of pollen morphology.

Doyle and Le Thomas (1996) stated that given the high level of morphological homoplasy in Annonaceae, only molecular analysis might be able to resolve higher-level relationships. The *Duguetia* alliance was addressed by van Zuilen (1996) with her cladistic analysis of *trn*L-F sequences, combined with morphological characters, favoring the inclusion of *Duguetia, Fusaea, Pachypodanthium,* and *Pseudartabotrys* as one clade. *Duckeanthus* and *Letestudoxa* were not included in her analysis.

Except for most seed characters, many of the morphological characters used in the above-mentioned analyses still are conventional characters in a Friesian vein (e.g., Fries, 1934, 1959), which have been subject to little recent critical revision. Moreover, regarding the reticulate nature of character expression in Annonaceae, the taxonomic level at which a phylogenetic analysis is performed determines the character choice. Contrasting with a family-wide phylogenetic analysis, an analysis at the tribal or genus group level requires different data matrices informative only for the particular group examined, as was elegantly shown by Johnson and Murray (1995) in their analysis of the tribe Bocageeae.

In this paper we address the phylogeny of the *Duguetia* alliance sensu Koek-Noorman et al. (1997) and Le Thomas et al. (1994), by conducting a cladistic analysis based on leaf, flower, fruit, and seed characters, many of which have not been used in cladistic analyses of Annonaceae before now. We provide the rationale for the recent submersion of

Table 1. Data matrix with taxa, characters, and character states used. The species abbreviations, as used in Figure 2, are in parentheses. *: African species of *Duguetia*. ?: character state unknown.

		1 2
		123456789012345678901234
Annona sericea Dunal	(Annser)	000000000000000000100001
Duckeanthus grandiflorus R. E. Fr.	(Ducgra)	000010200001001020110011
Duguetia argentea (R. E. Fr.) R. E. Fr.	(Dugarg)	020001000020000000000110
Duguetia asterotricha (Diels) R. E. Fr.	(Dugast)	010000000120020?00001110
Duguetia barteri (Benth.) Chatrou*	(Dugbar)	011001002120000101000110
Duguetia confinis (Engl. & Diels) Chatrou*	(Dugcon)	011001002120000101000110
Duguetia dilabens Chatrou & Repetur*	(Dugdil)	011001000020000101000110
Duguetia furfuracea (A.St.-Hil.) Benth. & Hook.f.	(Dugfur)	020001000020200100000110
Duguetia inconspicua Sagot	(Duginc)	010000000020000101000110
Duguetia lanceolata A. St.-Hil.	(Duglan)	020001000020200100000110
Duguetia neglecta Sandw.	(Dugneg)	010000001010000000000110
Duguetia quitarensis Benth.	(Dugqui)	020001000020000100000110
Duguetia riberensis Aristeg. ex Maas & Boon	(Dugrib)	011001001020000?00000110
Duguetia riparia Huber	(Dugrip)	010000000120000000000110
Duguetia spixiana Mart.	(Dugspi)	020001000021020000000110
Duguetia staudtii (Engl. & Diels) Chatrou*	(Dugsta)	011001000020000101000110
Duguetia uniflora (DC. ex Dunal) Mart.	(Duguni)	010000000020000110000110
Fusaea longifolia (Aubl.) Saff.	(Fuslon)	000110110121011020110001
Fusaea peruviana R. E. Fr.	(Fusper)	000110110121011020110001
Letestudoxa bella Pellegr.	(Letbel)	100100110011121120100011
Letestudoxa glabrifolia Chatrou & Repetur	(Letgla)	10010010001121?20100011
Letestudoxa lanuginosa Le Thomas	(Letlan)	100100100011221?2010????
Pseudartabotrys letestui Pellegr.	(Pselet)	100100000011101120101001

1—habit; 0 = tree/treelet, 1 = liana
2—trichomes; 0 = simple, 1 = stellate, 2 = lepidote (+ stellate)
3—leaf shape asymmetric; 0 = no, 1 = yes
4—secondary veins on upper side of leaf; 0 = flat to raised, 1 = impressed
5—secondary veins joining to form marginal vein; 0 = no, 1 = yes
6—mesophyll type; 0 = dorsiventral, 1 = isobilateral
7—histology of primary vein; 0 = phloem and/or sclerenchyma surrounding xylem body, 1 = phloem and/or sclerenchyma surrounding and intruding xylem body, 2 = phloem only abaxially accompanying xylem
8—curly trichomes; 0 = absent, 1 = present
9—inflorescence position: terminal on short axillary shoot; 0 = never, 1 = sometimes, 2 = always (ordered)
10—inflorescence: abnormal displacement of prophyll; 0 = absent, 1 = present
11—bracts; 0 = non-cucullate, scale-like, 1 = non-cucullate, foliaceous, 2 = cucullate
12—pedicel abruptly and distinctly widening into flowering receptacle; 0 = no, 1 = yes
13—flower color (in vivo); 0 = white to cream, 1 = yellow to orange, 2 = pink to red
14—fusion sepals; 0 = free or basally connate, 1 = subentirely connate, rupturing longitudinally, 2 = entirely connate, rupturing irregularly
15—petals velutinous; 0 = no, 1 = yes
16—stamen color (in vivo); 0 = white-cream-yellow, 1 = pink to red
17—stamens sclerified; 0 = no, 1 = only on adaxial side, 2 = on both sides
18—position of anther thecae; 0 = extrorse, 1 = latrorse
19—style; 0 = absent, 1 = present
20—styles coherent by interlocking papillae; 0 = no, 1 = yes
21—sepals persistent in fruit; 0 = no, 1 = yes
22—basal carpels fused with fruiting receptacle; 0 = no, 1 = yes
23—fruiting receptacle protruding between carpels; 0 = no, 1 = yes
24—direction of aril fibers; 0 = toward distal end of seed, 1 = toward proximal end of seed

Pachypodanthium into the synonymy of *Duguetia* (Chatrou, 1998). Species formerly known as *Pachypodanthium* will hereafter be referred to as "African species of *Duguetia*." A biogeographical question comprises whether the break-up of West Gondwana was a vicariance event for this subgroup as well as for most Annonaceae (Schatz & Le Thomas, 1993).

Data and Analyses

The data matrix includes 23 taxa and 24 characters (Table 1). All species of *Duckeanthus*, *Fu-*

saea, Letestudoxa, and *Pseudartabotrys* are included in the analysis. (For vouchers, see Appendix 1.) All 4 African species, and 11 Neotropical species of *Duguetia* are selected out of ca. 95 that constitute the genus. *Annona sericea* is included as outgroup taxon for two reasons. First, *Annona* is included in a clade that is directly linked to the pseudosyncarpous clade on the basis of sequence data (van Zuilen, 1996). Furthermore, of the genera that appear close to the pseudosyncarps in van Zuilen's analysis, *Annona* is the only genus with pseudosyncarpous fruits, and (often) with seeds provided with a rudimentary aril. Therefore, the scoring of characters 22–24 for the outgroup is enabled. The character set has been designed to incorporate independently evolving morphological and anatomical data from different plant parts. It comprises 17 binary characters and 7 three-state characters. Of the latter characters, only character 9 is quantitatively ordered, scoring for the relative abundance of the particular inflorescence position within a species. Although our objectives pertain to genera, the characters are scored at the species level. This allows the scoring of several characters with inconsistent character states within a genus. Characters 3, 6, 8, 9, 10, 11, 12, 13, 14, 16, and 18 are heterogeneous within a particular genus, while homogeneous within another. *Duguetia* possesses multiple states for all of these characters except for trichome character 8, while one of the other, nonmonotypic genera is scored uniformly. Scoring at the species level also permits us to address the question of the relationship between the Neotropical and African species of *Duguetia.* Bootstrap values of a previous phylogenetic analysis of *Duguetia* inspire little confidence in the clades found, nor in most of the sections of *Duguetia* as recognized by Fries (van Zuilen et al., 1995). Therefore, our choice of the 11 species for our analysis is such that they display the phenetic variation within the genus well. We decided to include *Letestudoxa lanuginosa* in the analysis, despite the fact that its fruits are unknown, and therefore characters 21–24 could not be scored. Inclusion may possibly allow more insight into the evolution of flower color (character 13), as *L. lanuginosa* has its flower color in common with only two species of *Duguetia* (*D. furfuracea* and *D. lanceolata*). The missing values for characters 21–24 do not present any problem for the analysis, as they are simply treated as uninformative.

Some of the characters in our analysis are straightforward morphological (or anatomical) ones. Other characters have hardly been documented, or are presented for the first time, with the following explanations.

Character 7: histology of the primary vein. Details hereon can be found in van Setten and Koek-Noorman (1986). In their survey of leaf anatomy of Annonaceae, *Duckeanthus* has not been taken into account. We sectioned leaf parts of *D. grandiflorus* according to the same methods as described in van Setten and Koek-Noorman (1986). The histology of the primary vein of *Duckeanthus* shows a pattern that is hitherto unknown in Annonaceae. The phloem only abaxially accompanies the xylem (Fig. 1A). This pattern is an autapomorphy of *Duckeanthus,* and we scored it as a separate character state.

Character 8: curly trichomes. Curly trichomes have been described for *Fusaea* (Chatrou & He, 1999) and *Letestudoxa* (Chatrou, 1998). In both genera these trichomes occur on the lower side of the leaves, on the petioles, and on the young twigs. In *Letestudoxa* they occur on the outer side of the calyx as well. Besides curly trichomes, normal straight trichomes occur as well in both genera.

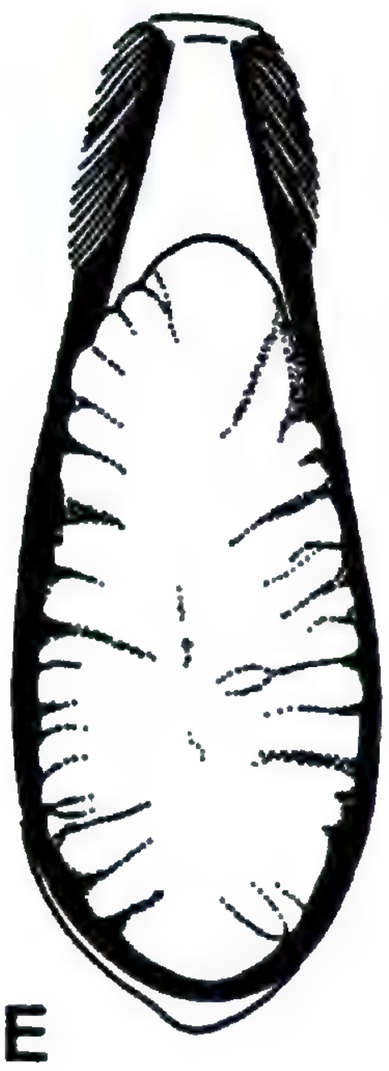
Character 9: position of inflorescence. Two African species of *Duguetia* exclusively have terminal inflorescences on reduced axillary leafy shoots. Le Thomas (1969) described them as axillary for *D. barteri.* Two Neotropical species of *Duguetia* (*D. neglecta* and *D. riberensis*) exhibit the same position of the inflorescence, though not in all cases. Hence, the latter two species have been scored as 1. The position of the inflorescences in other *Duguetia* species is terminal on leafy twigs, and never on reduced axillary leafy shoots.

Character 10: inflorescence, abnormal displacement of prophyll. This phenomenon has been described for inflorescences of *Fusaea* (Chatrou & He, 1999), and is also present in four species of *Duguetia.* Normally subsequent fertile prophylls alternate at angles of 180°. In *Fusaea, D. asterotricha, D. barteri, D. confinis,* and *D. riparia* the prophylls alternate at angles of ca. 90° only.

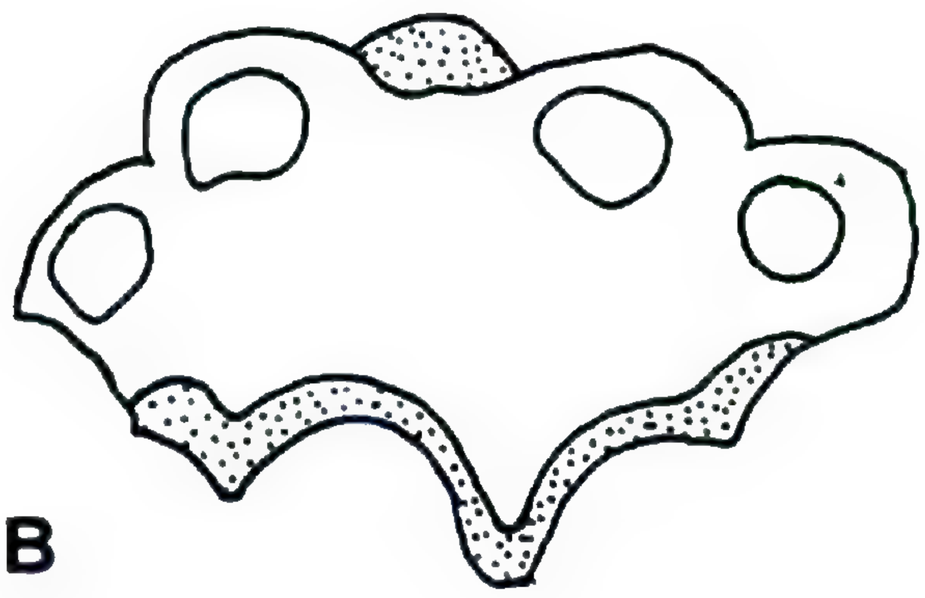
Character 11: shape of bracts. Cucullate bracts have been documented for *Fusaea* by Chatrou and He (1999), and have been found in all species of *Duguetia,* except for *D. neglecta,* which has foliaceous bracts.

Character 17: sclerified stamens. Van Heusden (1992) mentioned the occurrence of indurate (more or less lignified) stamens in *Duckeanthus, Fusaea, Letestudoxa,* and *Pseudartabotrys.* We made medial cross sections for at least 10 stamens per species, staining with Astra-blue and Safranin, to check for the occurrence of sclerenchyma. Two basic patterns were found: (1) sclerenchyma is either absent; or (2) sclerenchyma is present on both the entire inner

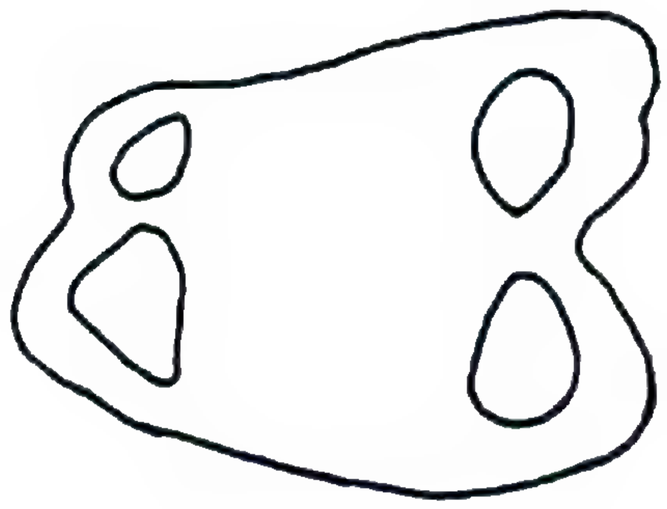
D



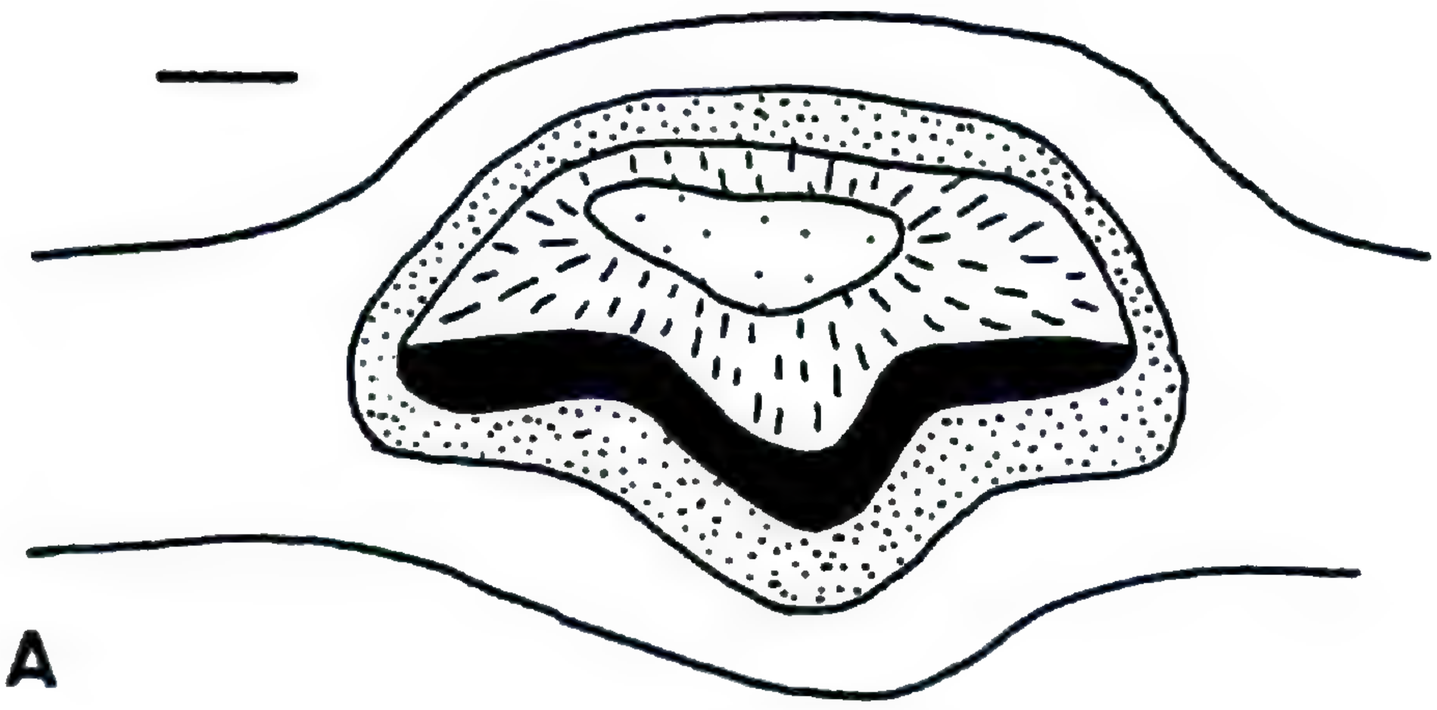
E



B



C



A

Sclerenchyma

Phloem

Xylem

Parenchyma

Figure 1. —A. Schematic drawing of transverse section through primary vein of *Duckeanthus grandiflorus*. B, C. Cross sections through stamens. —B. *Fusaea longifolia*, showing sclerified tissue (dotted region) and extrorse locules. —C. *Duguetia staudtii*, showing latrorse locules and no sclerification. D, E. Seeds transversely sectioned through raphe, showing direction of aril fibers. —D. Directed toward proximal end of seed: *Fusaea longifolia*. —E. Directed toward distal end of seed: *Duguetia confinis*. Scale bars: A–C = 0.1 mm; D, E = 1 mm.

side, as well as on the outer side between the thecae (Fig. 1B, C). Only *Duguetia uniflora* showed an intermediate pattern, with sclerenchyma found only on the inner side of the stamen, and absent between the thecae.

Characters 19 and 20: styles. For Annonaceae, the presence of a style is often difficult to judge. *Fusaea* has clearly distinct ovaries, styles, and stigmas. Its transition between ovary and style is indicated by a constriction, and by differences in epidermal outgrowths, and in shape in transverse section. The stigma can be discerned from the style by differences in color and epidermal outgrowths (Chatrou & He, 1999). The same pattern occurs in *Duckeanthus, Letestudoxa,* and *Pseudartabotrys.* As in *Fusaea,* the styles of *Duckeanthus* interlock by means of papillae. In those species of *Duguetia* examined, a clear distinction can be seen between the ovary and the apical part of the carpel, but subsequent transitions are absent. Therefore a style is considered to be absent in these genera, in spite of their presumed presence according to van Heusden (1992) and Doyle and Le Thomas (1996).

Characters 22 and 23: fruit type. Among fruits of different species of *Duguetia,* different degrees of fusion of the carpels occur. However, in all species the fruiting receptacle protrudes between the carpels by acropetal growth (Svoma, pers. comm. 1996). In fruits with a low degree of carpellary fusion this is very noticeable, especially when dried. Here, the surface of the receptacle shows shallow concavities in which the carpels are loosely positioned. Yet even in fruits with a high degree of carpellary fusion (e.g., *Duguetia furfuracea, D. barteri*) the protrusion of the receptacle between the carpels is discernible. Fruits of *Duckeanthus* and *Letestudoxa* have free, stipeless carpels, attached to the fruiting receptacle in shallow concavities, resulting in a functional syncarp (Schatz & Le Thomas, 1993) similar to those of *Duguetia.* An important difference between fruits of *Duckeanthus* and *Letestudoxa* on the one hand, and those of *Duguetia* on the other, is the position of the basal sterile carpels. In the latter genus, these basal carpels inseparably coalesce with the fruiting receptacle and constitute a proximal collar on the fruiting receptacle. In *Duckeanthus* and *Letestudoxa,* the basal, sterile carpels contribute to the functional syncarp and readily detach from the fruiting receptacle.

Character 24: direction of aril fibers. Arillate seeds are found in all species of the *Duguetia* alliance. The aril is considered to be rudimentary (van Setten & Koek-Noorman, 1992) as it covers considerably smaller parts of the seed than seen in species of the tribe Bocageeae. Among the *Duguetia* alliance, the aril develops from the base of the testa, distinguished from the other parts of the testa by its closely packed, long parallel cells (Garwood, 1995). We found that these rudimentary arils further assort into two types. The first type has the long, parallel cells directed toward the distal end of the seed, while in the second type they are directed proximally (Fig. 1D, E).

The data were analyzed using PAUP version 3.1.1 (Swofford, 1993). Heuristic searches for most parsimonious trees were performed by random stepwise addition with 100 repetitions, the Tree-Bisection-Reconnection (TBR) branch swapping algorithm, and the MULPARS and STEEPEST DESCENT options in effect. Only minimal trees were retained, and zero-length branches were broken down with the COLLAPSE option. The use of either the DELTRAN or the ACCTRAN optimization criterion produced identical tree topologies. Bootstrapping was performed with the TBR swapping algorithm, simple addition sequence, and 250 repetitions. The relative robustness of the clades was assessed additionally by performing a decay analysis (Bremer, 1988; Donoghue et al., 1992) for all clades of the strict consensus tree. Character evolution was analyzed using MacClade 3.04 (Maddison & Maddison, 1992).

Results and Discussion

Parsimony analysis of the data matrix in Table 1 resulted in 63 shortest trees of 49 steps. All 63 trees belong to one island of trees, with each tree connected to every other tree in the island through a series of trees, and each one differing from the next by a single rearrangement of branches (Maddison, 1991). The strict consensus tree has a consistency index (CI) of 0.65 and a retention index (RI) of 0.85 (Fig. 2). Bootstrap values are indicated above the nodes for each clade of the consensus tree that is maintained after bootstrap analysis. Bootstrap values ≥ 50 are given. Bootstrap values ≥ 70 are considered to be high. Our consensus tree satisfactorily meets the conditions under which bootstrap values ≥ 70 correspond to a probability of $\geq 95\%$ that the corresponding clade accurately reflects the true phylogeny (Hillis & Bull, 1993). Only the condition of internodal change of $\leq 20\%$ is not fully met: the basal nodes with bootstrap values of 95 and 88 both have an internodal change of 25% of the characters. Decay values are indicated below the nodes. We were unable to realize a decay analysis in which trees of three steps longer were retained. The large number of trees resulting

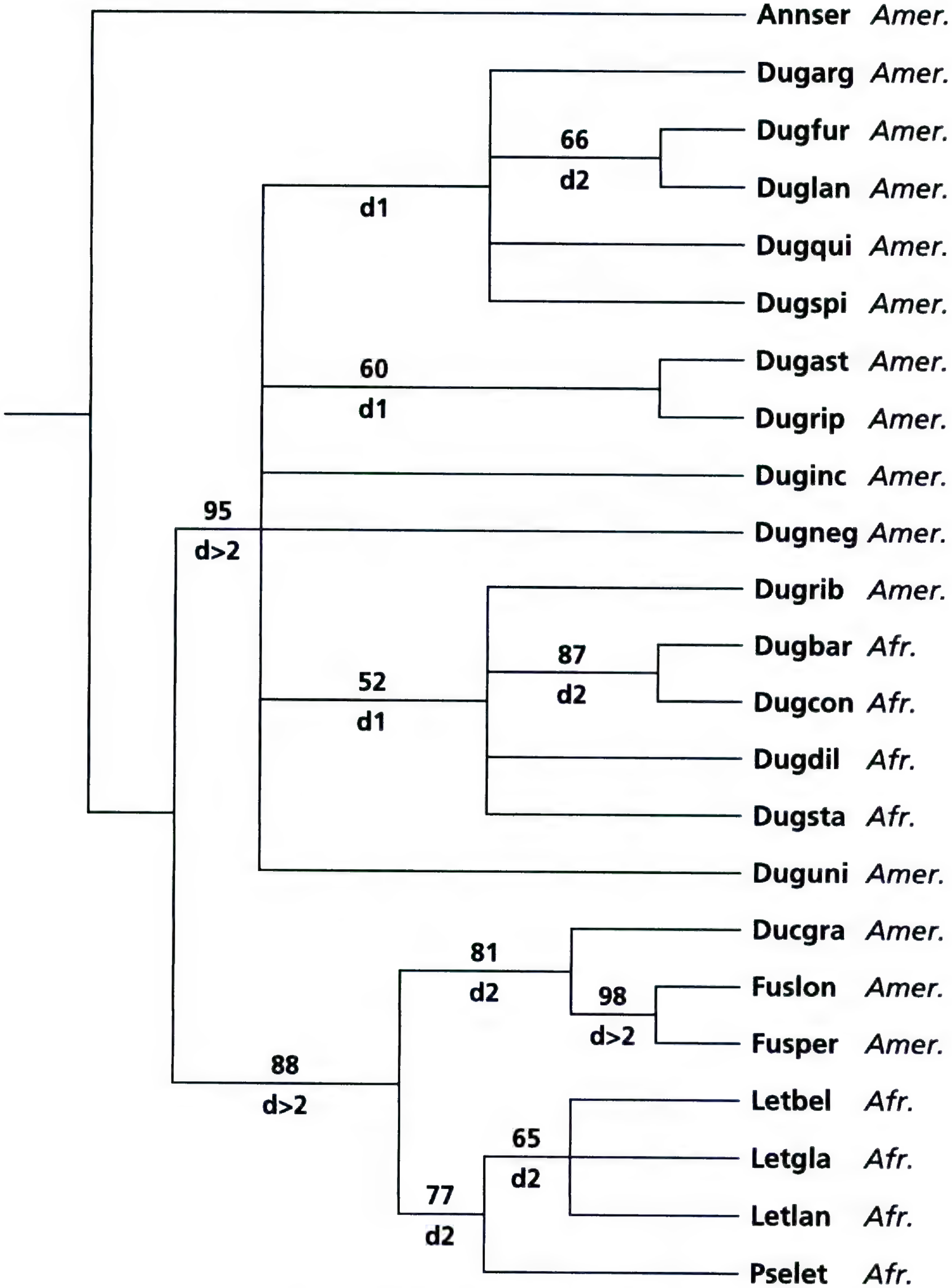


Figure 2. Strict consensus tree of 63 most parsimonious trees after analysis with outgroup rooting. Taxa are indicated with the first three letters of both generic name and epithet (see also Table 1). Bootstrap values are indicated above the nodes. Decay values are indicated below the nodes. *Amer.* and *Afr.* indicate American or African distribution of taxon.

Table 2. Synapomorphies for the combined *Duguetia-Fusaea* clade, and for the *Duguetia* clade and the *Fusaea* clade separately. The number of the character as described in Table 1 is included in parentheses.

Duguetia-Fusaea clade	*Duguetia* clade	*Fusaea* clade
No synapomorphies	(19) style absent (22) basal carpels fused with fruiting receptacle (23) fruiting receptacle protruding between carpels (24) aril fibers directed toward distal end of seed	(12) pedicel abruptly and distinctly widening into flowering receptacle (15) petals velutinous (17) stamens sclerified on both sides

from this search exceeded the maximum number of trees that PAUP can retain.

The high amount of synapomorphy, as expressed by the high RI, the high bootstrap values, and the highest decay values for (1) the clade formed by all species of *Duguetia* (*Duguetia* clade), and (2) the clade formed by *Duckeanthus*, *Fusaea*, *Letestudoxa*, and *Pseudartabotrys* (*Fusaea* clade), arouse high confidence in these clades. These results disapprove placement of all six genera into one clade or alliance (Doyle & Le Thomas, 1994, 1996; Koek-Noorman et al., 1997; Le Thomas et al., 1994), and support the distinction made among them by van Heusden (1992). The inclusion of *Afroguatteria*, *Disepalum*, and *Enicosanthellum* into the *Fusaea* group by van Heusden (1992), however, is contradicted by strong evidence, both from general morphology as well as from molecular evidence (Doyle & Le Thomas, 1994, 1996; Koek-Noorman et al., 1997; Doyle et al., 2000).

African species of *Duguetia* form a relatively distinct clade with *Duguetia riberensis* within *Duguetia*. Continued recognition of *Pachypodanthium* would have rendered *Duguetia* paraphyletic, and consequently *Pachypodanthium* species recently have been transferred to *Duguetia* (Chatrou, 1998). In the past *Pachypodanthium* has been considered to be different from *Duguetia* primarily on the basis of wood anatomy and palynology. Vander Wyk and Canright (1956) pointed out a difference in vessel density, being low for *Pachypodanthium* and high for *Duguetia*. Increased sampling has rendered the argument untenable (Ter Welle, pers. comm. 1997). Pollen grains of the African species of *Duguetia* possess an extremely reduced exine consisting of only spinules. Le Thomas et al. (1994) interpreted these spinules as homologous with verrucae in the Neotropical species, which also show varying exine reductions. Both pollen and wood indicate that *Duguetia*, despite its uniform appearance and its presumable monophyly (van Zuilen, 1996), remains a variable genus. Such is also demonstrated by our data matrix, with by far the largest part of the homoplasy deriving from *Duguetia*. The type of trichomes (character 2), asymmetric leaves (character 3), inflorescence position (character 9), and position of the thecae (character 18) represent features in our data matrix for which the African species seem anomalous. These aberrant character states are also encountered in a small subset of the Neotropical species of *Duguetia*. This reticulate nature of character expression, which was also found in a study of *Duguetia* leaf anatomy (Bakker & Visser, 1994), requires broader sampling of species; this is under way in a forthcoming analysis of *Duguetia* (Koek-Noorman & Maas, in prep.).

What are the character states that identify the combined *Duguetia-Fusaea* clade, the *Duguetia* clade, and the *Fusaea* clade? We traced all character states at the ingroup node, as well as at the internal nodes basal to the *Duguetia* clade and the *Fusaea* clade, respectively. The *Duguetia-Fusaea* clade is only characterized by traits that appear extensively within the Annonaceae, either by parallelism or by mosaic retention. Examples of these features include attributes such as trees, simple trichomes, symmetric leaves, free or basally connate sepals, and white to cream flower color. Thus, the *Duguetia-Fusaea* clade is not characterized by any synapomorphies (Table 2). The only character state that comes close to being synapomorphic is cucullate bracts, present in all *Duguetia* (except *D. neglecta*) and in *Fusaea*. However, it is one step more parsimonious to assume parallel evolution in *Duguetia* (and a subsequent loss in *D. neglecta*) and in *Fusaea*. The *Duguetia* clade and the *Fusaea* clade are characterized by four and three synapomorphies, respectively (Table 2).

To resolve relationships within the *Duguetia-Fusaea* clade we added some characters to the matrix that are novel, and which do not appear in the majority of other Annonaceae. This novelty does not

imply the change of one character state to another character state, and consequently these characters are difficult to polarize by outgroup comparison. This affects inflorescence character 10, implying a sympodial development of the inflorescence. The lack of any fertile inflorescence bracts in the tribe Bocageeae, and the presence of lower bracts that do not produce axillary buds in most *Xylopia* and many *Guatteria*, illustrate the paucity of sympodially developing inflorescences in Annonaceae. Character 20 for stylar papillae can only be scored for those few annonaceous species in which the carpels are provided with a style. Receptacular characters 22 and 23 require the presence of pseudosyncarpous fruits, which outside the group under study are only present in *Anonidium* and in the *Annona* group. Arillate character 24 can only be scored for a small group of genera where the seeds possess a rudimentary aril (van Setten & Koek-Noorman, 1992).

Considering this, outgroup comparison is difficult to use to polarize the set of characters at hand. Possible outgroup taxa may be selected from previous phylogenetic analyses. Doyle and Le Thomas (1994) found *Toussaintia* and the xylopioids connected basally to the pseudosyncarps, all of them together forming a monophyletic group. Van Zuilen (1996), based on limited sampling, however, found the *Duguetia-Fusaea* clade attached to a clade comprising *Uvaria, Uvariopsis, Isolona, Monodora, Annona,* and *Rollinia.* All these genera suffer from the comparative lack of the above-mentioned characters. The only exceptions are genera from the *Annona* group, which share sympodially developing inflorescences, pseudosyncarpous fruits, and rudimentary arils with the *Duguetia* alliance.

The character set as herein designed cannot establish the monophyly of the combined *Duguetia-Fusaea* clade, and cannot yield a corroborated positioning of the clade within the Annonaceae, as the selected characters obscure the choice of outgroup taxa. For the monophyly of the combined *Duguetia-Fusaea* clade we rely on Doyle and Le Thomas (1996) and Le Thomas et al. (1994).

Our study, as well as the cladistic analysis of the tribe Bocageeae (Johnson & Murray, 1995), shows that the search for new morphological characters with little generality, can be illuminative for analyses at low taxonomic level. The combined *Duguetia-Fusaea* clade (the "pseudosyncarps") shows no synapomorphies or unique combinations of characters. The challenge for future research will be to find synapomorphies that circumscribe more inclusive, monophyletic groups, to find new characters with more generality. Otherwise, it will be difficult to bring the phylogeny of Annonaceae beyond floating groups of genera with rather well resolved internal relationships.

Considering the geographical distibution of the taxa (Fig. 2), the pattern is straightforward, deriving from the break-up of Gondwana. Both the *Duguetia* clade and the *Fusaea* clade apparently existed before this event. Within the *Fusaea* clade, closer phylogenetic relationships correspond with geographical proximity. *Fusaea* and *Duckeanthus* evolved as the Neotropical representatives, and *Letestudoxa* and *Pseudartabotrys* as the African ones. We cannot address whether the biogeographic isolation of the African species of *Duguetia* corresponds with their constituting a separate clade within *Duguetia,* or whether some of the African species have closer sister-group relationships with Neotropical species. Analyses including more species of this genus, which will have to resolve this, are forthcoming.

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Appendix 1. Voucher specimens for taxa studied.

Annona sericea Dunal

FRENCH GUIANA. Saint-Maurice-Region de Saint Laurent, Piste d'Apatou, 8 Feb. 1990 (fr), *Cremers & Hoff 11256* (P, U, US). GUYANA. **East Berbice-Corentyne:** near Thompson's farm (Timehri), 31 Oct. 1981 (fl, fr), *Maas et al. 5932* (NY, U, WU). **Upper Demerara-Berbice:** vicinity of Mabura Hill, trail from Mabura Hill-Linden road to Demerara Landing, 26 Aug. 1988 (fl buds, fr), *Maas et al. 7144* (K, P, U, WU). SURINAM. **Marowijne:** along road near Mongotapoe, 10 June 1954 (fl buds, yfr), *Lindeman 6133* (NY, U). VENEZUELA. **Bolívar:** Street Alcabala Casa Blanca towards Isla Anacoco, 28 July 1981 (yfr), *Aymard et al. 313* (MO).

Duckeanthus grandiflorus R. E. Fr.

BRAZIL. **Amazonas:** São Felipe, upper Rio Negro, 9 Mar. 1944 (fr), *Baldwin 3584* (S, US); Camanáos, upper Rio Negro, 13 Oct. 1932 (fl), *Ducke 23904* (B, K, RB, S, US); right bank of Rio Negro, Ilha Tamanduá (locally Ilha Marajó), near Carapanã, 18 Oct. 1987 (fl), *Maas et al. 6772* (NY, U), *Maas et al. 6778* (NY, U); Ilha das Flores, Rio Negro, 17 Feb. 1959 (fr), *Rodrigues* (S).

Duguetia argentea (R. E. Fr.) R. E. Fr.

BRAZIL. **Amazonas:** km 124 of Manaus–Porto Velho Hwy., 25 Mar. 1974 (fr), *Campbell et al. P20915* (G, INPA, U); Carapanã, Upper Rio Negro, 17 Oct. 1987 (fl), *Maas et al. 6764* (NY, U). COLOMBIA. **Amazonas-Vaupés:** Río Apaporis, Jino Gojé, between Río Piraparaná and Río Popeyaká, Caño Unguyá, alt. 250 m, 3–11 Sep. 1952 (fl, fr), *García-Barriga 14366* (COL, US). VENEZUELA. **Amazonas:** San Carlos de Río Negro, 21 Mar.–17 Apr. 1981 (yfr), *Delascio C. et al. 9425* (VEN); Isla Sebástian, Río Casiquiare, between Boca and Chapazón, alt. 120 m, 31 Jan. 1980 (fl, fr), *Liesner & Clark 8939* (MO, U, VEN).

Duguetia asterotricha (Diels) R. E. Fr.

BRAZIL. **Amazonas**: km 118 of Manaus–Caracaraí Hwy., Mar. 1976 (fl), *D. Coêlho & Damião 767* (INPA); Reserva Florestal Ducke, alt. 80 m, 16 Jan. 1990 (fr), *Gentry & Revilla 69142* (U); ibidem, 18 Jan. 1990 (fl), *Gentry & Nelson 69219* (U); Manaus, 2 km from Tarumãzinho, 18 Nov. 1975 (fl), *O. P. Monteiro INPA53548* (INPA); km 70 of Manaus–Itacoatiara Hwy., 31 May 1994 (fl), *Webber 1477* (HUAM). PERU. **Loreto**: Mishuyacu, near Iquitos, Oct.–Nov. 1929 (fl), *Klug 86* (F, NY, US).

Duguetia barteri (Benth.) Chatrou

CAMEROON. **Centre-Sud:** bank of the Nyong River, 40 km SE of Yaoundé, alt. 550 m, 9 Nov. 1961 (fr), *Breteler 2013* (BR, K, P, WAG). **Littoral:** right bank of Ouem

River, near confluence with Sanaga River, 6 km SW of Masok, alt. 350 m, 4 Apr. 1965 (fl), *Leeuwenberg 5377* (BR, K, MO, WAG); Lombé, Tissongo, strip C, 26 July 1976 (fr), *McKey & Gartlan 139* (E). GABON. **Ogooué-Ivindo:** Ipassa, 10 km S of Makokou, small island in Ivindo River, alt. 500 m, 27 Nov. 1971 (fr), *Hladik 1835C* (US); Ipassa, 10 km from Makokou, Ivindo River, alt. 500 m, 14 Mar. 1975 (fl), *Hladik 2641* (P). NIGERIA. **Ondo:** Marbara River, Ibaji-Ojoku Reserve, 11 Mar. 1934 (fr), *Taylor 13* (FHO).

Duguetia confinis (Engl. & Diels) Chatrou

CAMEROON. **Centre-Sud:** ca. 16 km from Kribi, Ebolowa road, Bidou plantation, Kienké Forest Reserve, 3 Feb. 1969 (fr), *Bos 3844* (WAG); Bipindi, 1904 (fl), *Zenker 3195* (B, BR, E, G, GOET, L, M, MO, P, S, WU, Z). GABON. **Estuaire:** near Libreville, Apr. 1897 (fl), *Klaine s.n.* (P). **Moyen-Ogooué:** SW of Lambaréné, near Lake Ezanga, Conoco drilling site, alt. 20 m, 3 Feb. 1991 (fr), *McPherson 15195* (MO). **Nyanga:** 10 km on Maambi River, 7 Aug. 1992 (fl), *Wieringa & van de Poll 1360* (WAG). **Ogooué-Maritime:** Rabi, N of Shell camp, alt. 50 m, 26 Nov. 1994 (fl, fr), *Wieringa & van Nek 3290* (WAG).

Duguetia dilabens Chatrou & Repetur

GABON. **Centre-Sud:** forest reserve of Kienké Kribi, Ebolowa km 16, 5 Jan. 1968 (fr), *Bamps 1679* (BR). **Littoral:** Lombé, Tissongo, 7 Aug. 1976 (fr), *McKey & Gartlan 194* (K). **Ngounié:** new road from Mouila to Yeno, 5 km on either side of Kembele village, alt. 500 m, 20 July 1986 (fl, fr), *Thomas & Wilks 6510* (MO, P, WAG).

Duguetia furfuracea (A. St.-Hil.) Benth. & Hook.f.

BOLIVIA. **Santa Cruz:** Prov. Velasco, Serrania de Huanchaca, alt. 800 m, 3–4 Dec. 1987 (fl, fr), *Thomas et al. 5578* (U). BRAZIL. **Bahia:** 15–20 km from Andarai, along the road to Itaeté which branches E off the road to Mucugé, alt. 500–600 m, 13 Feb. 1977 (fl, fr), *Harley et al. 18634* (CEPEC, F, IPA, K, MO, NY, P, U, US). **Distrito Federal:** 20 km S of Brasília, on road to Belo Horizonte, alt. 700–1000 m, 26 Aug. 1964 (fl), *Irwin & Soderstrom 5572* (NY, S, SP, TEX). **Goiás:** Mun. Mineiros, 14 km E of turn-off for Mineiros, 1 Feb. 1986 (fl, fr), *Andersson & Hagberg 1635* (GB, U). **Mato Grosso:** Mun. Campo Grande, road from Campo Grande to Rochedo, 12 July 1969 (fl, fr), *Hatschbach & Guimarães 21837* (S, UC). **Minas Gerais:** Serra do Espinhaço, 6 km N of Gouvêia on road to Diamantina, alt. 1250 m, 10 Apr. 1973 (fl, fr), *W. R. Anderson et al. 8585* (F, MO, NY, RB, U, UB, US). **São Paulo:** Fazenda Hollambra, 35 km N of Campinas, alt. 600 m, 25 Feb. 1976 (fl), *Shepherd & Gibbs 11246* (K, MG, NY). PARAGUAY. **Amambay:** 14 km S of Bella Vista, alt. 250 m, 25 Mar. 1983 (fl), *Simonis et al. 197* (AAU, F, G, U).

Duguetia inconspicua Sagot

BRAZIL. **Amapá:** Rio Araguari, camp 13, 9 Oct. 1961 (fr), *J. M. Pires et al. 51633* (NY). **Pará:** basin of Rio Trombetas, 3 km up Rio Mapueira from Cachoeira Porteira, 30 May 1974 (fr), *Campbell et al. P22301* (NY, U). FRENCH GUIANA. Mt. Bellevue de l'Inini, alt. 700 m, 17 Aug. 1985 (fr), *de Granville et al. 7580* (B, CAY, P, U). GUYANA. Seballi Compartment, ca. 3 km S of Mabura, alt. 0–100 m, 1 Nov. 1991 (fl, fr), *Polak & Maas 379* (B, F, K, LZ, MO, U, ULM, WU). SURINAM. Nassau Mts., Marowijne River, alt. 430–520 m, 31 Dec. 1954 (fr), *Cowan & Lindeman 39044* (NY, S, U, US).

Duguetia lanceolata A. St.-Hil.

BRAZIL. **Minas Gerais:** Lagoa Santa, 8 Mar. 1865 (fl, fr), *Warming s.n.* (C, F, K, NY, P, S). **Paraná:** Sengés, 29 June 1910 (fl buds), *Dusén 9939* (GH, NY, S). **Santa Catarina:** Vargem Grande, Lauro Müller, alt. 350 m, 24 Oct. 1958 (fl), *Reitz & Klein 7483* (B, K, S). **São Paulo:** Mun. Brotas, Pepira-Mirim, Experimental Station of Mogi-Guaçu, Arboretum, alt. 500 m, 22 Sep. 1992 (fl), *Maas et al. 8043* (LZ, U, UEC, ULM, WU).

Duguetia neglecta Sandw.

GUYANA. Base of Mt. Makarapan, near rapids of Makarapan Creek, 15 Sep. 1988 (fr), *Maas et al. 7433* (B, BBS, F, MO, NY, U, VEN, WIS); Mabura Hill Nature Reserve, 25 Aug. 1990 (fl, fr), *Polak et al. 28* (U); Labbakabra Creek, Tiger Creek, Essequibo River, 26 Aug. 1937 (fl), *Sandwith 1214* (G, K, NY). SURINAM. Area of Kabalebo Dam project, along road between km 29 and 30, 1 Sep. 1980 (fl, fr), *Lindeman, Görts-van Rijn et al. 59* (F, K, NY, U).

Duguetia quitarensis Benth.

BOLIVIA. **Pando:** Río Madeira, 12 km above Abunã, 20 July 1968 (yfr), *Prance et al. 6213* (INPA, MG). BRAZIL. **Amazonas:** Mun. São Paulo de Olivença, near Palmares, 11 Sep. to 26 Oct. 1936 (fl), *Krukoff 8260* (A, BM, F, G, K, LE, MICH, MO, NY, S, U). **Pará:** Rio Cuminá-Mirim, 13 Dec. 1906 (fl), *Ducke MG7951* (BM, G, MG). COLOMBIA. **Meta:** Sierra de la Macarena, Caño Ciervo, alt. 600 m, 12 Jan. 1950 (fr), *Philipson et al. 2084* (COL, S, US). ECUADOR. **Napo:** La Joya de los Sachas, Parque Nacional de Yasuní, alt. 230 m, 8–15 July 1993 (fl), *Dik 54* (MO). GUYANA. Kanuku Mts., Puwib River, alt. 80–100 m, 13 Feb. 1985 (fl, fr), *Jansen-Jacobs et al. 184* (K, U, WIS). PERU. **Huánuco:** Pachitea, W of Puerto Inca, alt. 250–300 m, 14 Sep. 1982 (fr), *Foster 8778* (MO, U). **Loreto:** Prov. Maynas, Río Momon, 0–5 km from confluence with Río Nanay, alt. 100 m, 15 Nov. 1984 (fl, fr), *Maas et al. 6298* (AMAZ, K, U, USM, WIS). **Ucayali:** Prov. Coronel Portillo, Bosque A. von Humboldt, km 86 of road from Pucallpa to Tingo Maria, alt. 250 m, 2 Nov. 1984 (fl), *Maas et al. 6180* (U, USM). VENEZUELA. **Amazonas:** 5 km E of San Fernando de Atabapo, N bank of Río Orinoco, alt. 95 m, 4 May 1979 (fr), *Davidse et al. 17183* (MO, U).

Duguetia riberensis Aristeg. ex Maas & Boon

VENEZUELA. **Apure:** Distr. San Fernando, mouth of Río Arauca at its intersection with Río Orinoco, alt. 3 m, 14–15 May 1977 (fl), *Davidse & González 13219* (MO, U). **Bolívar:** Puerto Ordaz, San Félix, Apr. 1964 (fl), *Aristeguieta 5308* (HBG, U, VEN). **Guárico:** margins of Río Orituco, 5 km of Calabozo, Apr. 1963 (fl), *Aristeguieta & Tamayo 5087* (HBG, VEN).

Duguetia riparia Huber

BOLIVIA. **Pando:** S bank of Rio Abunã, between Cachoeiras Tres S and Fortaleza, 3–16 km above mouth, 18 July 1968 (fl buds), *Prance et al. 6132* (INPA, NY). BRAZIL. **Amazonas:** Reserva Forestal Ducke, km 26 of Manaus–Itacoatiara Hwy., 12 Oct. 1995 (fl), *Miralha, Maas et al. 308* (INPA, U). **Pará:** Belém, Reserva Mocambo, 8 Nov. 1995 (fl, fr), *Maas et al. 8360* (INPA, K, LZ, MG, MO, NY, U, ULM, WIS). COLOMBIA. **Caquetá:** Quebrada El Engaño, 26 Nov. 1991 (fl), *Duivenvoorden et al. 1669A* (U). FRENCH GUIANA. Oyapock River, Grande Roche, Saut Cafesoca, 31 July 1969 (fl), *Oldeman T.425* (CAY, P, U). SURINAM. S of Juliana top, 13 km N of Lucie River, alt. 350 m, 9 Aug. 1963 (fl), *Irwin et al. 54639* (B, F, G, M, NY, P, US).

Duguetia spixiana Mart.

BOLIVIA. **Beni:** Prov. Yacuma, SE of San Borja, alt. 350 m, 14 Sep. 1988 (fl), *Beck 16749* (LPB, U). BRAZIL. **Acre:** Mun. Senador Guiomard, km 33 of BR 317, 11

Oct. 1980 (fl), *Cid & Nelson 2834* (MG, NY, U). **Amazonas**: Mun. São Paulo de Olivença, near Palmares, basin of Rio Solimões, 11 Sep.–26 Oct. 1936 (fl, fr), *Krukoff 8402* (A, BM, F, G, K, LE, MICH, MO, NY, P, S, U). COLOMBIA. **Amazonas:** Quebrada Arara, 2 hours N of Leticia, near Río Amazonas, 27 Jan. 1971 (fl), *Croat 7546* (MO). ECUADOR. **Napo:** 4 km N of Coca, alt. 250 m, 15 Sep. 1986 (fl), *Palacios & Neill 1312* (MO, U). PERU. **Loreto:** 7 km SW of Iquitos, 30 July 1972 (fl, fr), *Croat 18572* (AAU, C, F, MO, NA, NY). **Ucayali:** Prov. Coronel Portillo, Carretera Alexandro, ca. 8 km W of Bosque von Humboldt, alt. 250 m, 2 Nov. 1984 (fl, fr), *Maas et al. 6183* (MO, U, USM, WIS).

Duguetia staudtii (Engl. & Diels) Chatrou

CAMEROON. **Littoral:** Douala-Edea Reserve, Tissongo Study Area, Transect B, June 1976 (fr), *Waterman & McKey 879* (U). CENTRAL AFRICAN REPUBLIC. Sangha Economique Prefecture, Ndakan, Gorilla study area, alt. 350 m, 1 Apr. 1988 (fr), *Harris & Fay 416* (MO, P). CONGO-BRAZZAVILLE. **Sangha:** W slope of Mt. Nabema, alt. 600–900 m, 15 Nov. 1991 (fr), *Thomas et al. 8877* (MO). GABON. **Woleu-Ntem:** Bitam, 20 Dec. 1933 (yfr), *Le Testu 9424* (BM, BR). IVORY COAST. **Néy:** 61 km N of Sassandra, W of Niapidou, alt. 100 m, 26 Jan. 1959 (fl, fr), *Leeuwenberg 2579* (BR, UC, Z). LIBERIA. **Bong:** Bong range, 15 Aug. 1962 (fl, fr), *Voorhoeve 1176* (B, BR, K). **Nimba:** Nimba Mts., alt. 500 m, 28 Feb. 1962 (fl), *Voorhoeve 891* (WAG). NIGERIA. **Calabar:** Oban, 9 Mar. 1959 (fl), *Talbot 1494* (BM, Z).

Duguetia uniflora (DC. ex Dunal) Mart.

BRAZIL. **Amazonas:** Rio Içana, 18 Nov. 1945 (fl), *Fróes 21407* (F, IAN, K, NY, US); Igarapé Tarumã-Açu, 30 km NW of Manaus, 8 Oct. 1990 (fl), *Miralha, Maas et al. 232* (U, ULM). **Roraima:** Rio Branco, at mouth of Rio Itapera, 8 June 1989 (fr), *Mori et al. 20424* (U). VENEZUELA. **Amazonas:** Tamatama, Upper Río Orinoco, alt. 125 m, 13 July 1942 (fr), *Ll. Williams 15859* (A, F, G, NY, RB, S, US, W).

Fusaea longifolia (Aubl.) Saff.

BOLIVIA. **Beni:** km 13 of Riberalta–Guayaramerin road, 19 Nov. 1989 (fl), *Daly et al. 6260* (MO, U). BRAZIL. **Amapá:** Mun. Oiapoque, 60 km SSE of Oiapoque, 1 Dec. 1984 (fl), *Mori et al. 17100* (NY, U). **Amazonas:** km 155 of Manaus–Itacoatiara Road, 16 Dec. 1974 (fr), *Gentry & Ramos 13345* (MO, U). **Rondônia:** Angustura, Rio Machado region, 5 Dec. 1931 (fr), *Krukoff 1537* (A, G, MICH, MO, NY, S, U). **Roraima:** Serra da Lua, 21 Jan. 1969 (fl buds), *Prance et al. 9367* (NY, U). COLOMBIA. **Antioquia:** Mun. Caucasia, road to Nechí, 14 km from Caucasia–Planeta Pica Road, Hacienda La Candeleria, alt. 50 m, 24 Nov. 1986 (fl), *Zarucchi & Cárdenas 4250* (COL, MO, NY, U). **Santander:** Puerto Parra-Campo Capote, 10 Dec. 1979 (fl, fr), *Rentería Arriaga et al. 2136* (COL, HUA); **Vaupés:** vicinity of Mitú, 20 May 1976 (fr), *Zarucchi 1607* (COL, GH, K). FRENCH GUIANA. Les Eaux Claires, near Crique Tortue, alt. 200 m, 10 Feb. 1993 (fl), *Maas et al. 8066* (U). GUYANA. **Upper Takutu-Upper Essequibo:** Kuyuwini landing, Kuyuwini River, alt. 200 m, 2 Feb. 1991 (fl), *Jansen-Jacobs et al. 2313* (U). PERU. **Loreto:** Jenaro Herrera, IIAP, Arboretum, alt. 120 m, 25 Oct. 1994 (fl buds, yfr), *Chatrou et al. 2* (AMAZ, U, USM, WU). **Madre de Dios:** Parque Nacional del Manú, 14 Oct. 1986 (fl buds) *Foster et al. 11824* (U).

Fusaea peruviana R. E. Fr.

PERU. **Amazonas:** Río Santiago, 2 km from Caterpiza, 19 Nov. 1979 (fr), *Huashikat 1326* (MO). **Huánuco:** W Sira Mts., 26 km S of Puerto Inca, Panguana Biological Field station, alt. 260 m, 1 Feb. 1993 (fl), *Listabarth & Vasquez 11-1293* (U), alt. 260–350 m, 20 Oct. 1994 (yfr), *Rainer 244* (U, WU). **Loreto:** Río Napo, Sucusari, Explornapo Camp, alt. 140 m, 3 Nov. 1994 (fl buds, fr), *Chatrou et al. 7* (AMAZ, NY, U, USM, WU); Andoas, Río Pastaza near Ecuador border, 15 Aug. 1980 (fl, fr), *Gentry et al. 29779* (F, MO, U); km 44 of Iquitos–Nauta road, 12 Dec. 1988 (fl, fr), *Vásquez & Jaramillo 11378* (MO, U, USM).

Letestudoxa bella Pellegr.

CAMEROON. **Centre-Sud:** near Kom Valley, 25 km E of confluent of Ntem River and Akom River, near Ebolowa, 5 Mar. 1970 (fl, fr), *Letouzey 10097* (BR, P). GABON. **Haut-Ogooué:** 70 km SSW of Moanda, alt. 730 m, 13 Oct. 1970 (fl buds), *Breteler 6877* (WAG); 42 km SE of Lambaréné, 3 Oct. 1968 (fl), *Breteler 5805* (WAG). **Ngounié:** Moucongo, 19 Oct. 1926 (fl), *Le Testu 6336* (BR, P). **Ogooué-Maritime:** Doudou Mts., ca. 35 km of Doussala, 27 Aug. 1985 (fl), *Reitsma 1432* (WAG); Rabi, 3 km S of platform Rabi 13, near old incat camp, alt. 50 m, 28 Sep. 1994 (fl), *Wieringa & Nzabi 2797* (WAG).

Letestudoxa glabrifolia Chatrou & Repetur

GABON. **Woleu-Ntem:** ca. 10 km on Tchimbélé–Assok road, alt. 630 m, 14 Sep. 1994 (fl buds), *Breteler 12858* (U, WAG); Tchimbélé, on lake border near dam, alt. 530 m, 26 Dec. 1989 (fr), *Wieringa 293* (WAG); Cristal Mts., 10 km on Tchimbélé–Kinguélé road, alt. 570 m, 24 Jan. 1983 (fr), *de Wilde 198* (WAG).

Letestudoxa lanuginosa Le Thomas

GABON. **Woleu-Ntem:** Nkout, 13 Oct. 1933 (fl), *Le Testu 9320* (BR, MO, P); Oyem, 7 May 1934 (fl), *Le Testu 9570* (P).

Pseudartabotrys letestui Pellegr.

GABON. **Estuaire:** E part of presidential reserve Wonga-Wongué, ca. 100 km S of Libreville, alt. 100 m, 3 Mar. 1983 (fr), *de Wilde et al. 890* (WAG). **Ogooué-Maritime:** Rabi-Kounga, E of Rabi, 29 Oct. 1991 (fl), *Breteler & Jongkind 10211* (WAG); near Rabi, Shell Oil Company's camp, 24 Nov. 1991 (fl), *McPherson 15564* (BR); Rabi, 1.5 km along pipeline to Echira, alt. 40 m, 24 Nov. 1994 (fl, fr), *Wieringa & van Nek 3273* (WAG); 22 km along a track leading in W direction into the Doudou Mts., alt. 150 m, 3 Dec. 1986 (fl), *de Wilde et al. 9136* (P, WAG); Rabi, Shell-Gabon, just E of the airstrip, alt. 80 m, 22 Jan. 1993 (fl, fr), *de Wilde & van der Maesen 10888* (WAG).

MOLECULAR SYSTEMATICS OF THE CHINESE *YINSHANIA* (BRASSICACEAE): EVIDENCE FROM PLASTID AND NUCLEAR ITS DNA SEQUENCE DATA[1]

Marcus Koch[2] *and Ihsan A. Al-Shehbaz*[3]

ABSTRACT

Species of the Chinese endemic genera *Yinshania, Hilliella,* and *Cochleariella* were originally placed in or closely associated with *Cochlearia.* A previous preliminary molecular study mainly on European *Cochlearia* and detailed morphological studies by us showed that this complex was not affined to *Cochlearia* s. str. Depending on the authority consulted, the number of taxa recognized in this complex ranged from 11 to 25 species in one to four genera. The present phylogenetic study is based on the analysis of the ITS (internal transcribed spacer regions of the nuclear ribosomal DNA) and the chloroplast *trn*L-intron sequences from 18 taxa. Resulting phylogenies were compared, and the results demonstrate that there are two different lineages. One lineage combines exclusively the highly polyploid taxa from *Hilliella* and *Cochleariella.* The second lineage includes the diploid taxa from *Yinshania.* However, incongruencies when nrDNA- and cpDNA-derived phylogenies were compared suggest hybridization between these two lineages. We followed a concept to combine all taxa of this complex into the genus *Yinshania.* Our results from phylogenetic analysis of nr and cpDNA support the association of *Yinshania* with *Cardamine* and *Rorippa,* rather than with *Cochlearia,* as was suggested by nearly all previous authors.

Key words: Brassicaceae, *Cochleariella, Hilliella,* molecular systematics, reticulate evolution, *Yinshania.*

Many authors follow Schulz (1936) and Schultze-Motel (1986) in dividing *Cochlearia* into the sections *Pseudosempervivum* Boiss., *Glaucocochleria* O. E. Schulz, *Cochlearia* (= *Eucochlearia* Prantl), and *Hilliella* O. E. Schulz. As shown by Koch et al. (1999a), however, this sectional classification is highly artificial. Section *Cochlearia* is widely distributed in Europe and the circumpolar region, whereas section *Glaucocochlearia,* which was raised to the generic rank by Pobedimova (1968), is restricted to southwestern Europe. The latter section consists of *C. glastifolia* L. and *C. megalosperma* (Maire) Vogt, as well as *C. aragonensis* Coste & Soulié, which was only recently included (Koch et al., 1996), although considered to be distantly related to the other two species (Koch et al., 1999a). Section *Cochlearia* consists of a species complex that demonstrates highly polymorphic chromosome numbers, and diverse ecological adaptation and geographic distributions. Morphological differences between phylogenetically sister taxa are often weak and poorly defined (Koch et al., 1996). Both sections *Cochlearia* and *Glaucocochlearia* are closely related to the genus *Ionopsidium* Rchb. (Koch et al., 1999a). Section *Pseudosempervivum,* which is centered in the Middle East and clearly unrelated to *Cochlearia,* is most closely related to *Masmenia* F. K. Mey. and *Noccaea* Moench (Koch et al., 1999a), both of which were segregated by Meyer (1973, 1979, 1991) from *Thlaspi* L. s.l.

The family of Brassicaceae is divided into several tribes and subtribes. Most of them are highly artificial, such as tribe Arabideae (Koch et al., 1999b) or Lepidieae (Zunk et al., 1996). Following classical tribal concepts, *Cochlearia* sect. *Pseudosempervivum,* sect. *Cochlearia,* and sect. *Glaucocochlearia* are members of tribe Lepidieae. Species originally assigned by Schulz (1923) to section *Hilliella* (*Yinshania, Hilliella,* and *Cochleariella*) were excluded from *Cochlearia* by Pobedimova (1970), who did not assign them to any genus. However, these species have recently been placed in three Chinese endemic genera, *Yinshania* Y. C. Ma & Y. Z. Zhao, *Cochleariella* Y. H. Zhang & Vogt, and *Hilliella* (O. E. Schulz) Y. H. Zhang, each of which was assigned to a different subtribe. The genus *Yinshania* (Ma & Zhao, 1979) was placed in subtribe

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Descurainiinae, tribe Sisymbrieae. The genus *Cochleariopsis* (Zhang, 1985), renamed as *Cochleariella* (Zhang & Cai, 1989), was placed in subtribe Cochleariinae, tribe Lepidieae, along with *Hilliella* s. str. (Zhang, 1986). Although a few studies on taxonomy, evolution, and origin of these genera (Zhang, 1987; Zhang & Xu, 1990; Zhang, 1993) have been made, nothing was said about their systematic position in relation to the remaining Asian and European taxa of *Cochlearia*. Zhang's (1987) division of *Yinshania* (excluding *Hilliella*) into two sections and two series and Zhao's (1992) classification of *Yinshania* (including *Hilliella*) into two sections and six series, were shown by Al-Shehbaz et al. (1998) to be highly artificial. In fact, one of the species assigned by Zhang (1987) to *Hilliella* and by Zhao (1992) to *Yinshania* was placed by Al-Shehbaz and Yang (1998) in the synonymy of *Cardamine fragariifolia* O. E. Schulz.

On the basis of a comprehensive morphological survey of *Yinshania*, *Hilliella*, and *Cochleariella*, Al-Shehbaz et al. (1998) reduced the latter two to synonymy of *Yinshania*, and concluded that there is no need for infrageneric subdivisions that do not reflect the phylogenetic relationships of this small genus of 13 species.

They also demonstrated that morphological characters previously used in the delimitation of species (e.g., density of papillae on the fruit valves, fruit shape, and seed number per fruit) are highly variable among and within different populations of the same species. Furthermore, differences in the compression of fruit (terete vs. latiseptate or angustiseptate) that were emphasized heavily by earlier authors (e.g., Schulz, 1936) in the delineation of genera were not found to be taxonomically useful in the *Yinshania* complex. As shown by Koch et al. (1999a), the placement of heavy emphasis on fruit compression has led to the artificial integration of several taxa into *Cochlearia* sect. *Pseudosempervivum* instead of *Thlaspi* s.l. In fact, terete and variously flattened fruits occur in numerous genera of the Brassicaceae, and in many cases this aspect of fruit morphology is taxonomically insignificant.

Morphological convergence and parallelism are widespread in the Brassicaceae (Dvorák, 1971; Meyer, 1973; Avetesian, 1983; Endress, 1992), and the dependence on such characters to construct phylogenies often leads to erroneous conclusions (Sytsma, 1990; Meyer, 1991). Recent molecular analyses (e.g., Warwick et al., 1992; Price et al., 1994; Mummenhoff & Koch, 1994; Zunk et al., 1996; Mummenhoff et al., 1997; Koch et al., 1998a, b; Koch et al., 1999a, b) have made significant contributions to a better understanding of the classification, generic delimitation, and phylogenetic relationships in the Brassicaceae. A preliminary study (Koch et al., 1999a) utilizing ITS nrDNA and cp *trn*L intron sequence data of four species of the *Yinshania* complex (including *Hilliella* and *Cochleariella*) clearly showed that the complex is unrelated to *Cochlearia*. In this analysis it has been shown that ITS and *trn*L intron sequence data provide sufficient sequence variation to distinguish significantly between *Yinshania* and *Cochleariella*/*Hilliella* accessions with both data sets. In order to gain a better insight of the phylogenetic relationships within this Chinese complex, we examined sequence variation of the internal spacer regions (ITS1 and ITS2) of nrDNA (Baldwin et al., 1995; Campbell et al., 1995) and of the cp *trn*L intron (Böhle et al., 1994; Gielly & Taberlet, 1994; van Ham et al., 1994; Koch et al., 1999a), and compared the derived molecular phylogenies with traditional concepts based on morphological data. This approach provided us with the opportunity to characterize species lineages and to analyze incongruencies between different data sets in order to test hypotheses of gene flow over lineages and chloroplast capture.

Materials and Methods

Plant material

Leaf material for DNA extraction was obtained from herbarium specimens (Table 1), most of which were provided and determined by Zhang Yu-hua (Institute of Materia Medica, Zhejiang Academy of Medicine, Hangzhou, People's Republic of China). We did not examine vouchers to verify Zhang's determinations. The samples represent a broad spectrum of species of *Yinshania*, *Hilliella*, and *Cochleariella*. *Cardamine flexuosa* With. and *Rorippa palustris* (L.) Besser served as the outgroups. The DNA sequences for the outgroups were obtained from Franzke et al. (1998).

DNA extraction, PCR-amplification, and sequencing

The total DNA for the outgroups was isolated from leaf tissues following the CTAB (cethyltriammoniumbromide) method of Doyle and Doyle (1987), as modified by Mummenhoff and Koch (1994). DNA extraction from herbarium material was performed in a mini preparation in Eppendorf reaction tubes from 50-µg dried tissue. Tissue was ground with sand and prewarmed 2X CTAB-buffer.

Table 1. Nomenclature, accession data, and GenBank sequence accession numbers for Chinese taxa under study. (Samples 1 through 21 were provided by Zhang, 22 through 26 by Al-Shehbaz.)

Specimen	Nomenclature according to Zhang	Nomenclature according to Al-Shehbaz et al. (1998)	Accession data (China)	Genbank accession code for ITS1 and ITS2 sequences, respectively	Genbank acc. code for the *trn*L intron sequence
1	*Yinshania qianningensis* Y. H. Zhang	Y. *acutangula* subsp. *wilsonii* (O. E. Schulz) Al-Shehbaz et al.	Sichuan, Erdaoqiao, Kangding, *Wu & Cai 9332*	AF100797/AF100798	AF100862
2	*Hilliella yixianensis* Y. H. Zhang	*Y. yixianensis* (Y. H. Zhang) Al-Shehbaz et al.	Anhui, Yixian, *Zhang 95001*	AF100817/AF100818	AF10863
3	*Hilliella sinuata* (Kuan) Y. H. Zhang & H. W. Li var. *qianwuensis* Y. H. Zhang	*Y. sinuata* subsp. *qianwuensis* (Y. H. Zhang) Al-Shehbaz et al.	Jiangxi, Qianwu, *Hu & Li 1761*	AF100833/AF100834	AF100869
4	*Hilliella shuangpaiensis* Y. H. Zhang	*Y. rupicola* subsp. *shuangpaiensis* (Z. Y. Li) Al-Shehbaz et al.	Hunan, Guanjisi, Nanyue, *Wu s.n.*	AF100815/AF100816	AF100865
5	*Yinshania acutangula* (O. E. Schulz) Y. H. Zhang (1)	*Y. acutangula* (O. E. Schulz) Y. H. Zhang subsp. *acutangula*	Neimongol, Zaogou, Baotou, Tomoteyouqi, *Zhao Yizhi s.n.*	AF100799/AF100800 AF100801/AF100802	AF100855
6	*Yinshania acutangula* (O. E. Schulz) Y. H. Zhang (2)	*Y. acutangula* (O. E. Schulz) Y. H. Zhang subsp. *acutangula*	Xizang, Xinrongxiang, Luolongxian, *Tan 88-3*	AF100803/AF100804	AF100856
7	*Yinshania henryi* (Oliver) Y. H. Zhang	*Y. henryi* (Oliver) Y. H. Zhang	Hubei, Shenlongjia, *Zhou 9107003*	AF100805/AF100806 AF100807/AF100808	AF100859
8	*Yinshania furcatopilosa* (K. C. Kuan) Y. H. Zhang	*Y. furcatopilosa* (K. C. Kuan) Y. H. Zhang	Hubei, Shenlongjia, *Zhou 9107001*	AF100809/AF100810 AF100811/AF100812	AF100860
9	*Hilliella sinuata* (K. C. Kuan) Y. H. Zhang & H. W. Li	*Y. sinuata* (K. C. Kuan) Al-Shehbaz et al. subsp. *sinuata*	Zhejiang, Sanfeng, Chengan, *Hong 966*	AF100831/AF100832	AF100868
10	*Hilliella alatipes* (Handel-Mazzetti) Y. H. Zhang & H. W. Li var. *micrantha* Y. H. Zhang	*Y. rivulorum* (Dunn.) Al-Shehbaz et al.	Hunan, Jianghua, *collector unknown 7300069*	AF100829/AF100830	AF100878
11	*Hilliella changhuaensis* Y. H. Zhang	*Y. lichuanensis* (Y. H. Zhang) Al-Shehbaz et al.	Anhui, Daping, Huangshan, *Wang s.n.*	AF100843/AF100844	AF100864
12	*Hilliella guangdongensis* Y. H. Zhang	*Y. lichuanensis* (Y. H. Zhang) Al-Shehbaz et al.	Guangdong, Renhua, *Deng 7298*	AF100813/AF100814	AF100880
13	*Hilliella lichuanensis* Y. H. Zhang	*Y. lichuanensis* (Y. H. Zhang) Al-Shehbaz et al.	Jiangxi, Taihe, *Lai 0592*	AF100845/AF100846 AF100847/AF100848	AF100871
14	*Hilliella rupicola* (D. C. Zhang & J. Z. Shao) Y. H. Zhang	*Y. rupicola* (D. C. Zhang & J. Z. Shao) Al-Shehbaz et al.	Anhui, Shitai, *Shao 835008*	AF100823/AF100824	AF100870
15	*Hilliella paradoxa* (Hance) Y. Z. Zhao	*Y. paradoxa* (Hance) Y. Z. Zhao	Sichuan, Beipei, Chongqing, *Wu & Cai 9221*	AF100827/AF100828	AF100866

Table 1. Continued.

Specimen	Nomenclature according to Zhang	Nomenclature according to Al-Shehbaz et al. (1998)	Accession data (China)	Genbank accession code for ITS1 and ITS2 sequences, respectively	Genbank acc. code for the *trn*L intron sequence
16	*Hilliella hunanensis* Y. H. Zhang	*Y. hunanensis* (Y. H. Zhang) Al-Shehbaz et al.	Hunan, Linxian, *Li et al. 646*	AF100825/AF100826	AF100879
17	*Hilliella fumarioides* (Dunn.) Y. H. Zhang & H. W. Li	*Y. fumarioides* (Dunn.) Y. Z. Zhao	Jiangxi, Wulaofeng, Lushan, *Xiong 6729*	AF100819/AF100820 AF100821/AF100822	AF100867
18	*Hilliella warburgii* (O. E. Schulz) Y. H. Zhang & H. W. Li (1)	*Y. fumarioides* (Dunn.) Y. Z. Zhao	Zhejiang, Ningbo, *Zheng 5572*	AF100839/AF100840	AF100874
19	*Hilliella warburgii* (O. E. Schulz) Y. H. Zhang & H. W. Li (2)	*Y. fumarioides* (Dunn.) Y. Z. Zhao	Zhejiang, Tiantai, *Hong s.n.*	AF100837/AF100838	AF100875
20	*Cochleariella zhejiangensis* (Y. H. Zhang) Y. Z. Zhao (1)	*Y. fumarioides* (Dunn.) Y. Z. Zhao	Zhejiang, Longquan, *collector unknown 233*	AF100851/AF100852	AF100877
21	*Cochleariella zhejiangensis* (Y. H. Zhang) Y. Z. Zhao (2)	*Y. fumarioides* (Dunn.) Y. Z. Zhao	Zhejiang, Tonglu, *Zhang 97001*	AF100841/AF100842 AF100849/AF100850	AF100876
22	*Y. furcatopilosa* (K. C. Kuan) Y. H. Zhang	*Y. furcatopilosa* (K. C. Kuan) Y. H. Zhang (A7)	Hubei, Shennog-Jia, *Shennong-Jia Expedition 21828* (PE)		AF100861
23	*Y. acutangula* (O. E. Schulz) Y. H. Zhang subsp. *acutangula*	*Y. acutangula* (O. E. Schulz) Y. H. Zhang subsp. *acutangula* (A11)	Hebei, Wu-An County, *He 21252* (PE)		AF100857
24	*Y. acutangula* (O. E. Schulz) Y. H. Zhang subsp. *acutangula*	*Y. acutangula* (O. E. Schulz) Y. H. Zhang subsp. *acutangula* (A12)	Neimongol, Sartchy, *David 2889* (P)		AF100858
25	unknown	*Y. lichuanensis* (Y. H. Zhang) Al-Shehbaz et al. (A20)	Jiangxi, Xing Zi County, *Lou 82103* (NAS)		AF100872
26	unknown	*Y. lichuanensis* (Y. H. Zhang) Al-Shehbaz et al. (A21)	Anhui, Huan Shan, Fu Xi, *Shao 810105* (PE)		AF100873

Organic extraction and DNA isolation from herbarium specimens followed Koch et al. (1996).

Double-stranded DNA of the complete ITS region, including the 5.8S rDNA gene, was amplified by 30 cycles of symmetric PCR using ITS primers initially designed by White et al. (1990) and modified by Mummenhoff et al. (1997). The 18F primer (5′-GGAAGGAGAAGTCGTAACAAGG-3′) is located at the 3′-end of the 18S rDNA gene, and primer 25R (5′-TCCTCCGCTTATTGATATGC-3′) is located at the 5′-end of the 25S rDNA. It has been reported that PCR selection of rDNA paralogues has occurred (Buckler et al., 1997). However, PCR selection might have only been important in high G+C content sequences (Buckler et al., 1997). Sequences from *Yinshania* and *Hilliella* (Koch et al., 1999a) are comparable in G+C content to sequences from *Gossypium*, in which PCR selection was probably weak (Wendel et al., 1995a). The resulting amplification product included ITS1, 5.8S rDNA, and ITS2. Only those PCR products were cloned into the pGEM-T-Easy cloning vector (PROMEGA) that showed a single band on ethidium bromide stained agarose gels. Two cloned ITS regions from two independent PCR reactions were sequenced (forward and reverse) with both amplification primers and two universal primers located in the flanking sites of the pGEM-T-Easy vector (t7-forward: 5′-gtaacgatttaggtgacactatcg-3, m13-reverse: 5′-agcggataacaatttcacacagga-3). This means that every single clone was sequenced four times to avoid sequence errors. The *trn*L (UAA) intron was amplified and sequenced by using the universal primer B49318 (5′-CGAAATCGGTAGACGCTACG-3′) located at the 3′-end of the *trn*L(UAA)5′-exon and A49855 (5′-GGGGATAGAGGGACTTGAAC-3′) located at the 5′-end of the *trn*L(UAA)3′-exon (Taberlet et al., 1991). The PCR profile used to amplify the *trn*L intron followed the following profile: hot start with 5 min. at 94°C, and 35 cycles of amplification (1 min. 94°C, 45 min. 50°C, 45 min. 72°C), final elongation step for 10 min. 72°C, and storage at 4°C. DNAs were cycle-sequenced using the Taq DyeDeoxy Terminator Cycle Sequencing Kit (ABI Applied Biosystems, Inc.). Products of the cycle sequencing reactions were run on an ABI 377XL automated sequencer (ABI Applied Biosystems, Inc.). Material from accession numbers 22–26 (Table 1) was only used for sequencing the *trn*L intron sequence, because amplification of the ITS regions failed totally.

DATA ANALYSIS

ITS data. Boundaries of the ITS regions and coding sequences were determined by comparison to those of *Sinapis alba* L. (Rathgeber & Capesius, 1989) and other Brassicaceae (Mummenhoff et al., 1997; Koch et al., 1999a). DNA sequences were aligned by hand. Parsimony analyses were performed with unordered Fitch parsimony and weighted parsimony with a transition:transversion weighting of 1.0:1.08 using PAUP version 3.1 (Swofford, 1993). The BRANCH-AND-BOUND algorithm was used to find maximally parsimonious trees. Bootstrap analysis (Felsenstein, 1985) was performed using 1000 replicates and the HEURISTIC search algorithm with the MULPARS option. We combined the GAPMODE=MISSING option with the coding of the gaps as additional presence/absence characters (Downie & Katz-Downie, 1996). This option decreases the number of equally parsimonious trees because of the redundancy resulting from having two sets of scored characters for the same indel events (Wojciechowski et al., 1993).

Evolutionary data are most often presented as a phylogenetic tree, the underlying assumption being that evolution is a branching process. However, empirical data is rarely ideal and often supports several trees instead of one unique tree. Hence, it makes sense to consider tree reconstruction methods that produce a tree if the given data heavily favor one tree over all others. Otherwise, methods that produce a more general graph that indicates different possible phylogenies are useful (Huson, 1998). One such method is the Split Decomposition introduced by Bandelt and Dress (1992) and its variations. In order to visualize conflicting phylogenetic signals, we analyzed all ingroup ITS sequences (see Fig. 6), using the software program SplitsTree version 1.0.3. (Huson & Wetzel, 1995 shareware ftp://ftp.uni-bielefeld.de/pub/math/splits/).

trn*L intron data.* DNA sequences were aligned by hand. Parsimony analysis was performed with PAUP (version 3.1; for options, see ITS data). Gaps were treated as additional unweighted binary characters. These gaps were coded using strict criteria: gaps must occur at the same position and have the same aligned length to be treated as homologous, and no splitting of one gap in two or more characters was performed (Koch et al., 1999a). Bootstrap analysis was performed as described above.

TRIBAL RELATIONSHIPS

To estimate the tribal affinity of *Yinshania*, we derived an ITS phylogeny with sequences from *Capsella rubella* Reut. (Koch et al., 1999b), *Arabidopsis thaliana* (L.) Heynh. (GenBank U43224), *Yinshania acutangula* (O. E. Schulz) Y. H. Zhang,

Barbarea vulgaris R. Br. (EMBO X98632), *Cardamine flexuosa* With. (Franzke et al., 1998), *Thlaspi arvense* L. (Koch et al., 1999a), *Cochlearia aestuaria* (Lloyd) Heywood (Koch et al., 1999a), *Brassica oleracea* L. (GenBank AF039994/AF040038), and *Sinapis alba* (EMBO X66325). DNA sequences were aligned by hand, and the alignment is shown in Figure 4. Alignment positions 109–160 were removed from subsequent analysis. Parsimony analyses were performed with unordered Fitch parsimony using PAUP version 3.1 (Swofford, 1993). The BRANCH-AND-BOUND algorithm was used to find maximally parsimonious trees with the GAPMODE=NEWSTATE option. Bootstrap analysis (Felsenstein, 1985) was performed using 1000 replicates and the HEURISTIC search algorithm employed with the MULPARS option. A decay analysis (Bremer, 1988) was performed in addition to the bootstrap approach, in order to assess the confidence that could be placed in the monophyly of clades. Decay indices (DI) were estimated according to Baum et al. (1994).

Results

ITS data

The total length of the alignment with accession numbers 1–21 and outgroups *Cardamine flexuosa* and *Rorippa palustris* is 464 bp, with 283 and 181 nucleotides in ITS1 and ITS2 spacer regions, respectively. The alignment required 36 (7.8%) gap positions, including the outgroups, and is shown in Figure 1. Sequence data were submitted to GenBank with accession numbers AF100793–AF100852 (Table 1). One third of these gaps is located between positions 117 and 133 in the ITS1. This region was completely excluded from subsequent analysis. Additionally, we excluded nucleotide position 465–467 and 473–484 from subsequent data analysis because of an ambiguous alignment (Fig. 1). Gaps from nucleotide positions 11–14 and 293–294 were treated as one single gap, respectively. The number of introduced gaps is comparable to a phylogenetic analysis of *Thlaspi* s.l. with 4.8% of the sites (Mummenhoff et al., 1997). In *Krigia* and outgroups, Kim and Jansen (1994) had to introduce gaps in 3.9% of the sites. Total lengths of ITS1 and ITS2 are nearly identical among the taxa surveyed, and vary between 447 bp (*Hilliella alatipes* (Hand.-Mazz.) Y. H. Zhang & H. W. Li var. *micrantha* Y. H. Zhang [= *Yinshania rivulorum* (Dunn) Al-Shehbaz et al.], accession no. 10) and 457 bp (*Cardamine flexuosa* and *Rorippa palustris*).

Phylogenetic analysis using Fitch parsimony, including the additional 0/1 matrix for the gap position, resulted in 24 most parsimonious trees (MPTs) with a length of 538 and a consistency index (CI) of 71.4% (66.9% if autapomorphies are excluded). Of the 264 variable nucleotide positions, 166 informative positions were in the ITS1 region (including 48 autapomorphies) and 98 in the ITS2 region (including 25 autapomorphies). Four out of 36 gap positions within the total sequence alignment are unique to a particular sequence (*Rorippa palustris* 2 gaps, acc. no. 3, and acc. no. 14). A calculation of the transition/transversion ratio for the MPTs revealed a ratio of 1.00:1.08. Therefore, we used a weighted parsimony approach with a character state weighting of 1.00:1.08 (transition:transversion), which resulted in one MPT that is also represented among the 24 MPTs from the Fitch parsimony with a consistency index of 67.7% (CI 57.9% if autapomorphies were excluded). We present the MPT from the weighted parsimony approach to demonstrate relative branch length (Fig. 2). Bootstrap values are provided from 1000 replicates using the weighted parsimony approach. For most taxa we identified only one ITS sequence type within a single specimen. For *Hilliella lichuanensis* Y. H. Zhang (acc. no. 13), *Cochleariella zhejiangensis* (Y. H. Zhang) Y. H. Zhang & R. Vogt (acc. no. 21), *Yinshania acutangula* (acc. no. 5), *Y. henryi* (Oliv.) Y. H. Zhang (acc. no. 7), and *Y. furcatopilosa* (K. C. Kuan) Y. H. Zhang (acc. no. 8), we found two very similar ITS sequences among the two clones sequenced. In the case of accession numbers 13, 5, 7, and 8, ITS1 and ITS2 regions differed by only a single site mutation. In the case of *C. zhejiangensis* (acc. no. 21), the two ITS types from the same individual differed by 15 mutations, which might indicate that two different ITS loci were cloned and sequenced. Both sequences clustered within the same clade. In *Hilliella fumarioides* (Dunn) Y. H. Zhang & H. W. Li (acc. no. 17), we detected two ITS sequences from a single individual that clustered in different clades (Figs. 2, 6). Both sequences differed by 76 mutations. The ITS data clearly support a separation of two clades consisting of *Yinshania* sensu Zhang (1987) and *Hilliella/Cochleariella.* Different accessions from one taxon (sensu Al-Shehbaz et al., 1998, refer to fig. 2) grouped at different positions in the case of *C. zhejiangensis* (acc. no. 20, not related to acc. nos. 18, 19, 21).

Using the taxonomic concept of Al-Shehbaz et al. (1998) and merging *Hilliella changhuaensis* Y. H. Zhang, *H. guangdongensis* Y. H. Zhang, and *H. lichuanensis* (acc. nos. 11, 12, 13, respectively) into *H. lichuanensis*, only accession numbers 11 and 13

```
 GAPS                    ****   *                                  *
       1                                                                60

C. FLEXUOSA   TCGTATCCTGCCCAAAA-AAGACCGAACCGCGGACCAAAGATCATCAACTCTGGTGAGCG
R. PALUSTRIS  TCGATCCTGTACATAAA-CAGAACGACCCGCGAACCAAAGATCATCACTCACGGTGGGCC
1             TCGATACCTGTCCAAAAACAGAACGACCCGCGAACCAAAGATCATCACTCGCGGTAGGCC
2             TCGATACCTGTCCCGAA-CAGAACGACCCGCGAACAACCGATCATCACTCTCGGTAGGCC
3             TCGATACCTGTCCCGAA-CAGAACGACCCGCGAACAATCGATCACCACTCTCGGTAGGCC
4             TCGATACCTGTCCCGAA-CAGAACGACCCGTGAACAATCGATCACCACTCTCGGTAGGCC
5a            TCGATACCTGTCCAAAACCAGAACGACCCGCGAACCAAAGATCATCACTCGCG-TAGGCC
5b            TCGATACCTGTCCAAAACCAGAACGACCCGCGAACCAAAGATCATCACTCGCG-TAGGCC
6             TCGATACCTGTCCAAAACCAGAACGACCCGCGAACCAAAGATCATCACTCGCG-TAGGCC
7a            TCGATACCTGTCCAAAAACAGAACGACCCGCGAACCAAAGATCATCACTCGCGGTAGGCC
7b            TCGATACCTGTCCAAAAACAGAACGACCCGCGAACCAAAGATCATCACTCGCGGTAGGCC
8a            TCGATACCTGTCCAAAAACAGAACGACCTGCGAACCAAAGATCATCACTCGCGGTCGGCC
8b            TCGATACCTGTCCAAAAACAGAACGACCCGCGAACCAAAGATCATCACTCGCGGTCGGCC
9             TCGATACCTGTCCCGAA-CAGAACGACCCGCGAACAATCGATCACCACTCTCGGTAGGCC
10            TTGTCTTAACCTGGAAA-CAGAACGACCCGAGAACGTTGAAACATCACTCTCGGTGGGCC
11            TCGATACCTGTCCCGAA-CAGAACGACCCGCGAACAACCGATCATCACTCTCGGTAGGCC
12            TCGTAACCTG----GAAACAGAACGACCCGAGAACGTTGAAACATCACTCTCGGTGGGCC
13a           TCGATACCTGTCCCGAA-CAGAACGACCCGCGAACAACCGATCATCACTCTCGGTAGGCC
13b           TCGATACCTGTCCCGAA-CAGAACGACCTGCGAACAACCGATCATCACTCTCGGTAGGCC
14            TCGTAACCTG----GAAACAGAACGACCCGAGAACGTTGAAACATCACTCTCGGTGGGCC
15            TCGATACCTGTCCCGAA-CGGAACGACCCGCGAACAACCGATCATCACTCTCGGTAGGCC
16            TCGTAACCTG----GAAACAGAACGACCCGAGAACGTTGAAACATCACTCTCGGTGGGCC
17a           TCGATACCTGTCCCGAA-CAGAACGACCCGCGAACAATCGATCACCACTCTCGGTAGGCC
17b           TCGATACCTGTCAAAAA-CAGAACGACCCGAGAACGATTAATCATCACTCTCGGTAGGCC
18            TCGATACCTGTCCAAAA-CAGAACGACCAGCGAACTTTCGATCAACACTCCCGGTAGGCC
19            TCGATACCTGTCCAAAA-CAGAACGACCAGCGAACTTTCGATCAACACTCCCGGTAGGCC
20            TCGATACCTGTCCAAAA-CAGAACGACCCGCGAACCGAAGATCATCACTCGCGGTAGGCC
21a           TCGATACCTGTCCAAAA-CAGAACGACCAGCGAACTTTCGATCAACACTCCCGGTAGGCC
21b           TCGATACCTGTCCAAAA-CAGAACGACCAGCGAACTTTCGATCAACACTCCCGGTAGGCC
```

Figure 1. Alignment of ITS1 and ITS2 regions from *Yinshania*, *Cochlearia*, and *Hilliella*, as well as outgroups *Cardamine flexuosa* and *Rorippa palustris*. Characters excluded from data analysis are marked (Δ). Gap positions 1–4 were treated as a single binary 0/1 character. Taxon enumeration follows Table 1.

```
                      *                         *                *     **********  *    *
    61                                                           [del. Δ: 117 - 133]                               160
                                                                 ΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔ

    GGTTTCATAACTTGAGATCGTGCCTGCCGAATCCGTGGTTTCGCCGAACAATCCTTACCGGGAGCTTAATCTCTGTTTGGATTGTGCGCGTTGCTTCCGG
    GGTTTCTTAGCCGAGATC-GTGCCTGCCGAATCCGTGGTTTCGCGAGCAATCCTTACCGGGAGCTCT-ATCTCGGTTTGGGTTGTGCGCGTTGCTTCCGG
1   GGTTTCTTAGCCGATTCC-GTGCCTGCCGAGTCCGTGGTTTCGCGTATTGTCCCGGGCGAGAGCTCT-ATCTCGGT-CGTGTCGTGCGCGGTAGTTCCGG
2   GGATTCCCGACGGTCTCC-GCGCCTACCGATTCCGTGGTTTCGCGCACGTTTCAGCCAAGG---TCTTGCCTCGGC-GGGTTCGTGCGCGCAGCTTCCGG
3   GGTTCTTAAACAGTCCTC-GCGTCTACCGATTCCGTGGCTTCGCGTATGTCTCCATCGAGA--GTTTTACCTCGGT-GGGTTCATGCACGCGGCTTCCGG
4   GGTTCCTAAACAGTCCTC-GCGTCTACCAATTCCGTGGTTT-GAGCACGTCTACATGAGAG---TTTTACCTCGGT-GGATTCATGCACGCGGCTTCCGG
5a  GGTTTCTTAGCCGATCCCGGTGCCTGCCGTGTCCGTGGTTTCGCGTATTGTCCCGGGCGAGAGCTCT-ATCTCGGT-CGTGTCGTGCGCGTAGCTTCCGG
5b  GGTTTCTTAGCCGATCCCGGTGCCTGCCGTGTCCGTGGTTTCGCGTATTGTCCCGGGCGAGAGCTCT-ATCTCGGT-CGTGTCGTGCGCGTAGCTTCCGG
6   GGTTTCTTAGCCGATCCCGGTGCCTGCCGTGTCCGTGGTTTCGCGTATTGTCCCGGGCGAGAGCTCT-ATCTCGGT-CGTGTCGTGCGCGTAGCTTCCGG
7a  GGTTTCTTAGCCGATCCC-GTGCCTGCCGAGTCCGTGGTTTCGCGTATTGTCCCGGGCGAGAGCTC-AATCTCGGT-CGTGTCGTGCGCGTAGCTTCTGG
7b  GGTTTCTTAGCCGATCCC-GTGCCTGCCGAGTCCGTGGTTTCGCGTATTGTCCCGGGCGAGAGCTC-AATCTCGGT-CGTGTCGTGCGCGTAGCTTCTGG
8a  GGTTTCTTAGCCGATCCC-GTGCCTGCCGAGTCCGTGGTTTCGCGTATTGTCCCGGGCGAGAGCTC-TATCTCGGT-CGTGTCGTGCGCGTAGCTTCCGG
8b  GGTTTCTTAGCCGATCCC-GTGCCTGCCGAGTCCGTGGTTTCGCGTATTGTCCCGGGCGAGAGCTC-TATCTCGGT-CGTGTCGTGCGCGTAGCTTCCGG
9   GGTTCCTAAACAGTCCTC-GCGTCTACCGATTCCGTGGCTTCGCGTATGTCTCCATCGAGA--GTTTTACCTCGGT-GGGTTCATGCACGCGGCTTCCGG
10  GGTTTCTTAGCTGATTTC-GTGCCTACCGATTCCGTGGTTATGCGTTCGTCACCGGCCCAG-----TT---TCGGT-TGGATCATACGCATAGCTTCCGG
11  GGGTTCCAGACGGTCCCC-GCGCCTACCGATTCCGTGGTTTCGCGCACGTTTCAGCCAAGG---CCTCGCCCCGGC-TGGTTCGTGCGCGCAGCTTCCGG
12  GGTATCTTAGCTGATTTC-GTGCCTACCGATTCCGTGGTTATGCGTTCGTCACCGGCCCAG-----TT---TCGGT-CGGATTGTACGCATAGCTTCCGG
13a GGGTTCCAGACGGTCCCC-GCGCCTACCGATTCCGTGGTTTCGCGCACGTTTCAGCCAAGG---CCTCGCCTCGGC-TGGTTCGTGCGCGCAGCTTCCGG
13b GGGTTCCAGACGGTCCCC-GCGCCTACCGATTCCGTGGTTTCGCGCACGTTTCAGCCAAGG---CCTCGCCTCGGC-TGGTTCGTGCGCGCAGCTTCCGG
14  GGTTTCTTAGCTGATTTC-GTGCCTACCGATTCCGTGGTTATGCGTTTGTCACCGGCCCAG-----TT---TCGGT-TGGATCATACGCATAGCTTCCGG
15  GGGTTCCCGACGGTCCCC-GCGCCTACCGATTCCGTGGTTCCGCGCACGTTTCAGCCAAGG---TCTCGCCTCGGC-TGGTTCGTGCGCGCAGCTTCCGG
16  GGTTTCTTAGCTGATTTC-GTGCCTACCGATTCCGTGGTTATGCGTTCGTCACCGGCCCAG-----TT---TCGGT-TGGATCATACGCATAGCTTCCGG
17a GGTTCCTAAACAGTCCTC-GCGTCTACCGATTCCGTGGCTTCGAGCACGTCTCCATCAGAG---TTTTACCTCGTT-GGGTTCATGCACGCGGCTTCCGG
17b GGTTTCTTAGCTGATCCC-GTGCTTGCCGATTCCGTGGTTTCGCGTATCGTCCCTTCAAGA---TTTTATCTCGGC-TGGGTCATGCGTGTTGCTTCCGG
18  GGTTTCTTGACAGATCTC-GTGCCTACCGGATCCGTGGTCTCGCTCACGTTTCCCT-TAAAATGTTTTACATTGGT-TGGATCATGGCGCGGCTTCCGG
19  GGTTTCTTGACAGATCTC-GTGCCTACCGGATCCGTGGTCTCGCTCATGTTTCCCTCAAAAATGTTTTACATTGGT-TGGATCATGGCGCGGCTTCCGG
20  GGTTTCTTAGCCGATCCC-GTGCCTACCGATTCCGTGGTTCATGTTTGGCTTCGTC-AGTA-TTCATTACAT-GGC-GAAGTCATGCATGTTTCCTCCGG
21a GGTTTCTTGACAGATCTC-GTGCCTACCGGATCCGTGGTCTCGCTCATGTTTCCCTCAAAAATGTTTTACATTGGT-TGGATCATGGCGCGGCTTCCGG
21b GGTTTCTTGACAGATCTC-GTGCCTACCGGATCCGTGGTCTCGCTCATGTTTCCCTCAAAAATGTTTTACATTGGT-TGGATCATGGCGCGGCTTCCGG
```

Figure 1. Continued.

```
                *                                                                                   *
     161                                                                                          260

     ATATCACAAAACCACGGCACGAAAAGTGTCAAGGAACATGCAATTGAACAGACAGCCTTCGCCTCCCCGGAGACGGTGTGTGTGCGGATCCTGCGCTGCG
     ATATCACAAAACCCCGGTACGAAAAGTGTCAAGGAACATGCAATTGAACAGCCAGCCTTCGCCTCCCCGGTCACGGTGCGTGTGCGGTTNCTGTNCTNCG
1    ATTTCACCAAACCACGGCACGAAAAGTGTCAAGGAACATGCAACAGAACGGCCACGGTTCGCCTCCCCGGATACGGTGTGAGTGCGGATGTTGTGCTACG
2    ATATCACAAAACCCCGGCACGAAAAGTGTCAAGGAACATTGCACCGATCGGCCTGCCTTCGCCGCCCCGGAGACGGTGCGCGTGCGGATGCAGCGCCGCG
3    ATATCACAAAACCACGGCACGAAAAGTGTCAAGGAACATCGAACCGATCGGCCGGCCTTCGCCGCCCCGGAGACGGTGCGCGTGCGGTTGCCGG-CTGCA
4    ATATCACAAAACCACGGCACGAAAAATGTCAAGGAACATCGAACCGATCGGCCTGCCTTCGCCGCCCCGGAGACGGTGCGCGTGCGGAAGCCGTGCTGCA
5a   ATATCACCAAACAC-GGCACGAAAAGTGTCAAGGAACATGCAACAGAACGGCCAGCCTTCGCCTCCCCGGATACGGTGTGAGTGCGGATGTTGTGCTGCG
5b   ATATCACCAAACAC-GGCACGAAAAGTGTCAAGGAACATGCAACAGAACGGCCAGCCTTCGCCTCCCCGGATACGGTGTGAGTGCGGATGTTGTGCTGCG
6    ATATCACCAAACAC-GGCACGAAAAGTGTCAAGGAACATGCAACAGAACGGCCAGCCTTCGCCTCCCCGGATACGGTGTGAGTGCGGATGTTGTGCTGCG
7a   ATATCACCAAACCACGGCACGAAAAGTGTCAAGGAACATGCAACAGAACGGCCAGCCTTCGCCTCCCCGGATACGGTGTGAGTGCGGATGTTGTGCTGCG
7b   ATATCCCCAAACCACGGCACGAAAAGTGTCAAGGAACATGCAACAGAACGGCCAGCCTTCGCCTCCCCGGATACGGTGTGAGTGCGGATGTTGTGCTGCG
8a   ATATCACCAAACCACGGCACGAAAAGTGTCAAGGAACATGAAACGGAACGGCCAGCCTTCGCCTCCCCGGATACGGTGTGAGTGCGGATGTTGTGCTGTG
8b   ATATCACCAAACCACGGCACGAAAAGTGTCAAGGAACATGAAACAGAACGGCCAGCCTTCGCCTCCCCGGATACGGTGTGAGTGCGGATGTTGTGCTGTG
9    ATATCACAAAACCACGGCACGAAAAGTGTCAAGGAACATCGAACCGATCGGCCGGCCTTCGCCGCCCCGGAGACGGTGCGCGTGCGGATGCCGTGCTGCA
10   ATATCACAAAACCCCGGCACGAAAAGTGTCAAGGAACATTCAACTAAACGGCCTGCTTTCGCCAACCCGGAAACGGTGTTTGTTCGGAAACAGTGTTGCA
11   ATATCACAAAACCCCGGCACGAAAAGTGTCAAGGAACATTGAACCGATCGGCCTGCCTTCGCCGCCCCGGAGACGGTGCGCGTGCGGATGCAGNNNNNNN
12   ATATCACCAAACCCCGGCACGAAAAGTGTCAAGGAACATTCAACTAAACAGCCTGCTTTCGCCAACCCGGAGACGGTGTTTGTTCGGAAGCAGTGCTGCA
13a  ATATCACAAAACCCCGGCACGAAAAGTGTCAAGGAACATTGAACCGATCGGCCTGCCTTCGCCGCCCCGGAGACGGTGCGCGTGCGGATGCAGCGCCGCG
13b  ATATCACAAAACCCCGGCACGAAAAGTGTCAAGGAACATTGAACCGATCGGCCTGCCTTCGCCGCCCCGGAGACGGTGCGCGTGCGGATGCAGCGCCGCG
14   ATATCACCAAACCCCGGCACGAAAAGTGTCAAGGAACATTCAACTAAACGGCCTGCTTTCGCCAACCCGGAGACGGTGTTTGTTCGGAAGCAGTGCTGCA
15   ATATCACAAAACCCCGGCACGAAAAGTGTCAAGGAACATCGAACCGATCGGCCTGCCTTCGCCGCCCCGGAGACGGTGCGCGTGCGGATGCAGCGCCGCG
16   ATATCACCAAACCCCGGCACGAAAAGTGTCAAGGAACATTCAACTAAACGGCCTGCTTTTGCCAACCCGGAGACGGTGTTTGTTCGGAAGCAGTGCTGCA
17a  ATATCACAAAACCACGGCACGAAAAGTGTCAAGGAACATCGAACCGATCGGCCTGCCTTCGCCGCCCCGGAGACGGTGCGCGTGCGGAAGCCATGCTGCA
17b  ATATCACAAAACCCCGGCACGAAAAGTGTCAAGGAACATGCAACTAAACAGCTTGCGTTCGCCTACCCGGAGACGGAGACGGTGTGGTGCGGATGCTGCA
18   ATATCACAAAACCCCGGCACGAAAAGTGTCAAGGAACATGCAACTAAACAGCCTGTCTTCGCCGCCCCGGAGACGGTGTGCGCGCGGATGCAGTGCTGCT
19   ATATCACAAAACCCCGGCACGAAAAGTGTCAAGGAACATGCAACTAAACAGCCTGTCTTCGCCGCCCCGGAGACGGTGTGCGCGCGGATGCAGTGCTGCT
20   ATTTCACCAAACCACGGCACGAAAAGTGTCAAGGAACATTCAACTAAACGGCCTGCTTTCGCCAACCCGGAGACGGTGTTTGTTCGGAAGCAGTGCTGCA
21a  ATATCACAAAACCCCGGCACGAAAAGTGTCAAGGAACATGCAACTAAACAGCCTGTCTTCGCCGCCCCGGAGACGGTGTGCGCGCGGATGCAGTGCTGCT
21b  ATATCACAAAACCCCGGCACGAAAAGTGTCAAGGAACATGCAACTAAACAGCCTGCCTCCGCCGCCCCGGAGACGGTGTGCGCGCGGATGCAGTGCTGCT
```

Figure 1. Continued.

```
                      *         **                 *                  *
    261                 283ITS2                                                              360

    ATCTAAAGTCTATCGTCGTCCCTCTCATCCTTCTTAGGACGTGGGACGGAAGCTGGTCTCCCGTGTGTTA-CCGCACGCGGTTGGCCCAAATCCGAGCTA
    ATCTAAAGTCTATCGTCGTCCCTCTCATCCTTC-TCGGATATGGGACGGAAGCTGGTCTCCCTTGTGTTA-CCGCATGCGGTTGGCCGAAATCCGATCTA
1   ATCTAAAGTCTATCGTCGTCCATTTCATTCCTATAAGGATCCGGGACGGAAGCTGGTCTCCCGTGTGTTA-CCGCACGCGGTTGGCCAAAATCCGAGCAA
2   ATCTAAAGTCTACCGTCGTCCCCTACATCCCCGAAGGATACGGGACGGAAGCTGGTCTCCCGTGTGGTA-CCGCACGCGGTTGGCCGAAATCCGAGCCA
3   ATCTAAAGTCTACCGTCGTCCCC-ACATCCCCTAAGGGTACGGGACGGAAGCTGGTCTCCCGTGTGGTA-CCGCACGCGGTTGGCCGAAATCCGAGCTA
4   ATCTAAAGTCTACCGTCGTCCTCTACATCCCCCAAGGATACGGGACGGAAGCTGGTCTCCCGTGTGGTA-CCGCACGCGGTTGGCCGAAATCCGAGCTA
5a  ATCTAAAGTTTATCGTCGTCCCCTTCATCCTTT-GCGGATACGGGACGGAAGCTGGTCTCCCGTGTGTTA-CCGCACGCGGTTGGCCAAAATCCGAGCAA
5b  ATCTAAAGTCTATCGTCGTCCCCTTCATCCTTT-GCGGATACGGGACGGAAGCTGGTCTCCCGTGTGTTA-CCGCACGCGGTTGGCCAAAATCCGAGCAA
6   ATCTAAAGTCTATCGTCGTCCCCTTCATCCTTT-GCGGATACGGGACGGAAGCTGGTCTCCCGTGTGTTA-CCGCACGCGGTTGGCCAAAATCCGAGCAA
7a  ATCTAAAGTCTATCGTCGTCCCCTTCATCCTT--GCGGATACGGGACGGA-GCTGGTCTCCCGTGTGTTA-CCGCACGCCGTCGGGCAAAATCCGAGCAA
7b  ATCTAAAGTCTATCGTCGTCCCCTTCATCCTT--GCGGATACGGGACGGA-GCTGGTCTCCCGTGTGTTA-CCGCACGCCGTCGGGCAAAATCCGAGCAA
8a  ATCTAAAGTCTATCGTCGTCCCCTTCATCCTTT-GCGGATACGGGACGGAAGCTGGTCTCCCGTGTGTTA-CCGCACGCGGTTGGCCAAAATCCGAGCAA
8b  ATCTAAAGTCTATCGTCGTCCCCTTCATCCTTT-GCGGATACGGGACGGAAGCTGGTCTCCCGTGTGTTA-CCGCACGCGGTTGGCCAAAATCCGAGCAA
9   ATCTAAAGTCTATCGTCGTCCCC-CCATCCTCTCGAGGATATCGGACGGAAGCTGGTCTCCGGTGTGTTA-CCGCACGCGGTTGGCCAAAATCCGAGCTA
10  AGGTAAAGTCTATCGTCGTCCCC-CCATCCTCTCGAGGATATCGGACGGAAGCTGGTCTCCCGTGCGTTA-CCGCACGCGGTTGGCCAAAATCCTAGCTA
11  NNNNAAAGTCTACCGTCGTCCCC-ACATCCCC-CGAGGATACGGGACGGAAGCTGGTCTCCCGTGTGGTA-CCGCACGCGGTTGGCCGAAATCCGAGCCA
12  ATGTAAAGTCTATCGTCGTCCCC-CAATCCTCTCGAGGATATCGGACGGAAGCTGGTCTCCCGTGTGTTA-CCGNACGCGGTTGGCCAAAATCCGAGCTA
13a ATCTAAAGTCTACCGTCGTCCCC-ACATCCCC-CGAGGATACGGGACGGAAGCTGGTCTCCCGTGTGGTA-CCGCACGCGGTTGGCCGAAATCCGAGCCA
13b ATCTAAAGTCTACCGTCGTCCCC-ACATCCCC-CGAGGATACGGGACGGAAGCTGGTCTCCCGTGTGGTA-CCGCACGCGGTTGGCCGAAATCCGAGCCA
14  ATGTAAAGTCTATCGTCGTCCCC-CCATCCTCTCGAGGATATCGGACGGAAGCTGGTCTCCCGTGTGCCATCCTCTCGAGGATGGCCAAAATCCTAGCTA
15  ATCTAAAGTCTACCGTCGTCCCC-ACATCCCC-CGAGGATATCGGACGGAAGCTGGTCTCCCGTGTGTTA-CCGCACGCGGTTGGCCGAAATCCGAGCCA
16  ATGTAAAGTCTATCGTCGTCCCC-CCATCCTCTCGAGGATATCGGACGGAAGCTGGTCTCCCGTGTGTTA-CCGACGCCGGTTGGCCAAAATCCTAGCTA
17a ATCTAAAGTCTACCGTCGTCCCC-ACATCCCCTAAGGATACGGGACGGAAGCTGGTCTCCCGTGTGGTA-CCGCACGCGGTTGGCCGAAATCCGAGCTA
17b ATCTAAAGTCTACCGTCGTCCCC-NNATCCTTTCGAGGATGCTGGACGGAAGCTGGTCTCCCGTGTGTTA-CCGCATGCGGTTGGCCAAAATCCGAGCAA
18  AAATAAAGTCTATCGTCGTCCCC-ACATCCCTTCGAGGACACGGGACGGAAGCTGGTCTCCCGTGTGGTA-CCGCACGCGGTTTGCCAAAATCCGAGCTA
19  AAATAAAGTCTATCGTCGTCCCC-ACATCCCCTCGAGGACACGGGACGGAAGCTGGTCTCCGGTGTGTTA-CCGCTCGCGGTTTGCCAAAATCCGAGCTA
20  ATGTAAAGTCTATCGTCGTCCCC-CCATCCTCTCGAGGATATCGGACGGAAGCTGGTCTCCCGTGTGTTA-CCGCACGCGGTTGGCCAAAATCCTAGCTA
21a AAATAAAGTCTATCGTCGTCCCC-ACATCCCCTCGAGGACACGGGACGGAAGCTGGTCTCCGGTGTGTTA-CCGCTCGCGGTTTGCCAAAATCCGAGCTA
21b AAATAAAGTCTATCGTCGTCCCC-ACATCCCCTCGAGGACACGGGACGGAAGCTGGTCTCCCGTGTGGTA-CCGCACGCGGTTGGCCAAAATCCGAGCTA
```

Figure 1. Continued.

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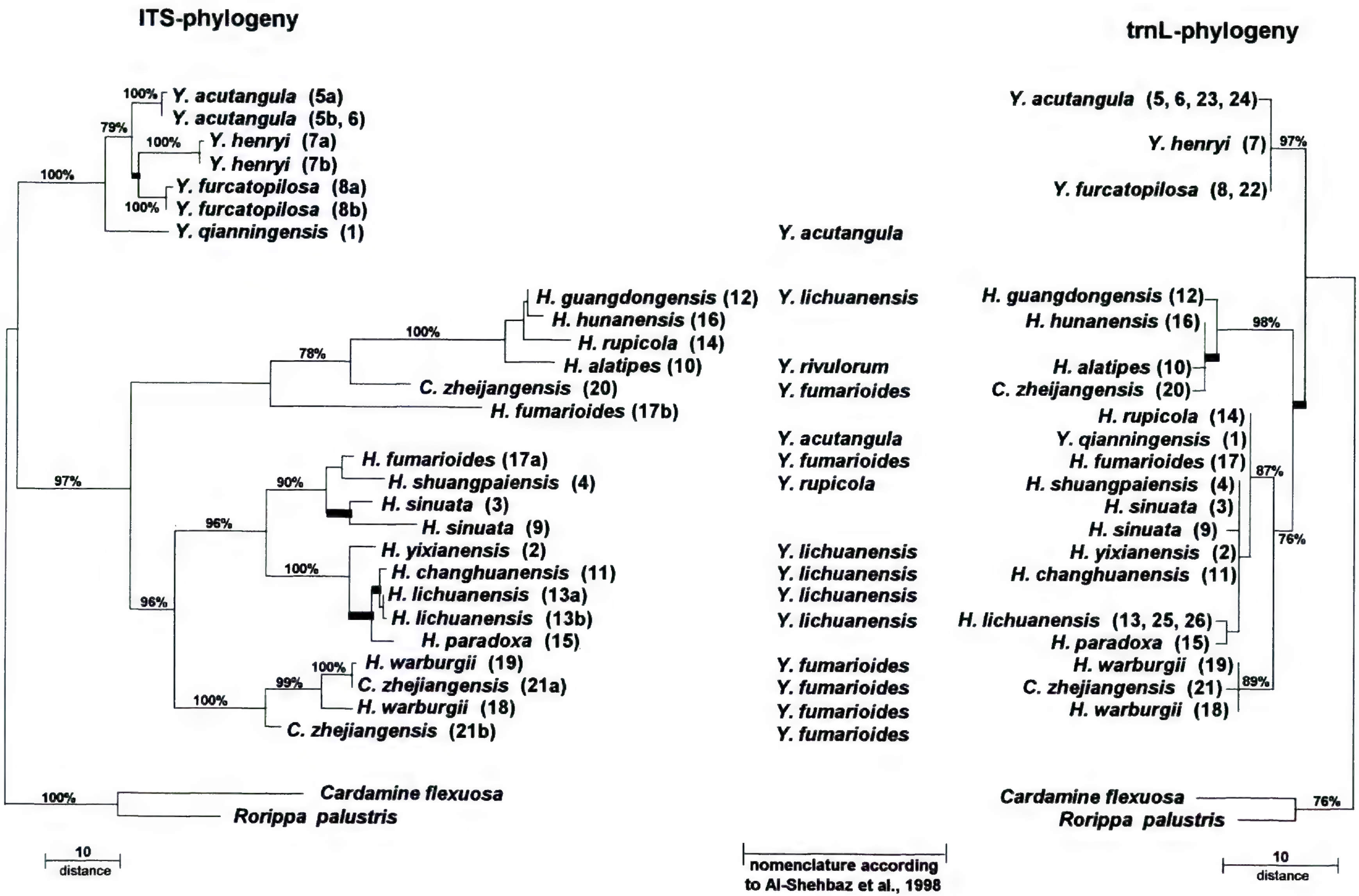
        *     *                                     *     **                    *        *  *
     361                                                                                             460

     AGG-ACGCCGAGAGCGTCACGACATGCGGTGGTGAACTAAAGCCTGT-TCGTA--TCGTCGGTCGTTCTTGTCTATAAGCTCTCGATGACCCAAAATCCT
     AGG-ACGCCGAGAGCGTCACGACATGCGGTGTTGAACTAAAGCCTCT-TCGTCAGTCGTCGGTCGTTCTTGTCCGGAAGCTCTCGATGACCCAAAGTCCT
1    AGG-ACGCCGGGAGCGTCCCGACATGCGGTGGTGTGTGCAAGCCTCGATCATA--TCGTCGGCCGCTCCTGTCCGAAAGCTCTAGATGACCCAAAGTCTT
2    AGG-ACGTAGGGAGCGCCCCGACATGCGGTGGTGAATCCAAGCCTCG-TCATA--TCGTCGGCCGCTTCTGTCCGGACGCTCTAGTTGACCCAAAGTCCT
3    AGG-GCGCCAGGAACGTCCCGACATGCGGTGGTGAATCCAAGCCTCG-TCATA--CGGTCGGCCGCTCCTGTCCGGATGCTCTCGATGACCCAAAGTTCT
4    AGG-GCGCCAGGAACGTCCCGACATGCGGTGGTGAATCCAAGCCTCG-TCATA--CGGTCGGCCGCTCCTGTCCGGATGCTCTCGATCACCCAAAGTTCT
5a   AAGGACGCC-GGAGCGTCCCGACATGCGGTGGTGTGTGCAAGCCTCTATCATA--TCGTCGGTCGCTCCTGTCCGAAAGCTCTAGATGACCCAAAGTCTT
5b   AAGGACGCC-GGAGCGTCCCGACATGCGGTGGTGTGTGCAAGCCTCTATCATA--TCGTCGGTCGCTCCTGTCCGAAAGCTCTAGATGACCCAAAGTCTT
6    AAGGACGCC-GGAGCGTCCCGACATGCGGTGGTGTGTGCAAGCCTCTATCATA--TCGTCGGTCGCTCCTGTCCGAAAGCTCTAGATGACCCAAAGTCTT
7a   AAGGACCCG-GGAGCGTCCCGACATGCGGTGGTGTGTGCAAGCCTCTATAATA--TCGTCGGCCGCTCTTGTCCG-AAGCTCTA-AT-ACCCAAAGT-TT
7b   AAGGACCCG-GGAGCGTCCCGACATGCGGTGGTGTGTGCAAGCCTCTATAATA--TCGTCGGCCGCTCTTGTCCG-AAGCTCTA-AT-ACCCAAAGT-TT
8a   AAGGACGCCGGGAGCGTCCCGACATGCGGTGGTGTGTGCAAGCCTCTATCATA--TCGTCGGCCGCTCCTGTCCGAAAGCTCTTGATGACCCAAAGTCTT
8b   AAGGACGCCGGGAGCGTCCCGACATGCGGTGGTGTGTGCAAGCCTCTATCATA--TCGTCGGCCGCTCCTGTCCGAAAGCTCTTGATGACCCAAAGTCTT
9    AGG-GCGCCAGGAACGTCCCGACATGCGGTGGTGAATCCAAGCCTCG-TCATA--CCGTCGGGCGTTTCTGTCCGGATGCTTTTATGACCCAAAGTTTT
10   AGG-ATGCCAGGAGCGTCTTGACATGCGGTGGTGAATTCAATTCTCG-TCAAA--TCGTCAGTCGTTTCGGTCCGAAAGCTCTTGATGACCCAAAGTCCT
11   AGG-ACGCAGGGAGCGCCCCGACATGCGGTGGTGAATCCAAGCCTCG-TCATA--TCGTCGGCCGCTCCTGTCCGGACGCTGTAGACGACCCAAAGTCCT
12   AGG-ATGCCAGGAGCGTCTTGACATGCGGTGGTGAATTCAATTCTCG-TCAAA--TCGTCAGTCGTTTCGGTCCGAAAGCTCTTGATGACCCANAGTCCT
13a  AGG-ACGCAGGGAGCGCCCCGACATGCGGTGGTGAATCCAAGCCTCG-TCATA--TCGTCGGCCGCTCCTGTCCGGATGCTCTAGACGACCCAAAGTCCT
13b  AGG-ACGCAGGGAGCGCCCCGACATGCGGTGGTGAATCCAAGCCTCG-TCATA--TCGTCGGCCGCTCCTGTCCGGATGCTCTAGACGACCCAAAGTCCT
14   AGG-ATGCCAGGAGCGTCTTGACATGCGGTGGTGAATTCCATTCTCG-TCAAA--TCGTCAGTCGTTTCGGTCCGAAAGCTCTTGATGACCCTTTGTCCT
15   AGG-ACGCAGGGAGCGCCCCGACATGCGGTGGTGAATCCAAGCCTCG-TCATA--TCGTCGGCCGCTCCTGTCCGGACGCTCTAGACGACCCAAAGTCCT
16   AGG-ATGCCAGGAGCGTCTTGACATGCGGTGGTGAATTCAATTCTCG-TCAAA--TCGTCAGTCGTTTCGGTCCGAAAGCTCTTGATGACCCAAAGTCCT
17a  AGG-GCGCCAGGAACGTCCCGACATGCGGTGGTGAATCCAAGCCTCG-TCATA--CGGTCGGCCGCTCCTGTCCGGATGCTCTCGATGACCCAAAGTTCT
17b  AGG-ACGCCTGGCGCGTCTCGACATGCGGTGGTGGATTCAATTCTCG-TCAAA--TTGTCGGTCGTTTCTGTCCGGAAGCTCTCGATGACCCAAAGTCCT
18   AGG-ACGCTAGGAGCCGTCTCGCATGCGGTGGTGAATCCAAGCCTCG-TCATA--TCGTCGGCCGCTCCTGTCCGGATGCTCTCGATGACCCGATTCGTT
19   AGG-ACGCTGGGAGCCGTCTGGCATGCGGTGGTGAATCCAAGCCTTG-TCATA--TCGTCNNCCCGCTCTGTTCCGATGCTTTNGATGACCCGATTCGTT
20   AGG-ATGCCAGGAGCGTCTTGACATGCGGTGGTGAATTCAATTCTCG-TCAAA--TCGTCAGTCGTTTCGGTCCGAAAGCTCTTGATGACCCAAAGTCCT
21a  AGG-ACGCTGGGAGCCGTCTGGCATGCGGTGGTGAATCCAAGCCTTG-TCATA--TCGTCNNCCCGCTCTGTTCCGATGCTTTNGATGACCCGATTCGTT
21b  AGG-ACGCTAGGAGCGTCTCGACATGCGGTGGTGAATCCAAGCCTCG-TCATA--TCGTCGGCCCGCTCTGTCCGGACGCTCTAGACGACCCAAAGTCCT
```

Figure 1. Continued.

		gaps: ITS1	ITS2
	466		
	CAAA	11110111111	1111001000111
	CAAA	11110101111	1101001011111
1	CAAA	11111101011	1111001100111
2	CAAC	11110101011	1111001000111
3	CAAC	11110101010	0111001000111
4	CAAC	11110100011	1111001000111
5a	CAAA	11111011001	1101010100111
5b	CAAA	11111011001	1101010100111
6	CAAA	11111011001	1101010100111
7a	CAAG	11111101011	1000110100000
7b	CAAG	11111101011	1000010100000
8a	TCAA	11111101011	1101011100111
8b	TCAA	11111101011	1101011100111
9	CAAC	11110101011	0111001000111
10	CAAC	11110101011	0111001000111
11	CAAC	11110101011	0011001000111
12	CAAC	00001101011	0111001000111
13a	CAAC	11110101011	0011001000111
13b	CAAC	11110101011	0011001000111
14	CAAC	00001101011	0111101000111
15	CAAC	11110101011	0011001000111
16	CAAC	00001101011	0111001000111
17a	CAAC	11110101011	0111001000111
17b	CAAC	11110101011	0111001000111
18	CAAC	11110101011	0111001000111
19	CAAC	11110101011	0111001000111
20	CAAC	11110101011	0111001000111
21a	CAAC	11110101011	0111001000111
21b	CAAC	11110101011	0111001000111

Figure 1. Continued.


ITS-phylogeny
Y. acutangula (5a)
Y. acutangula (5b, 6)
Y. henryi (7a)
Y. henryi (7b)
Y. furcatopilosa (8a)
Y. furcatopilosa (8b)
Y. qianningensis (1)
H. guangdongensis (12)
H. hunanensis (16)
H. rupicola (14)
H. alatipes (10)
C. zheijangensis (20)
H. fumarioides (17b)
H. fumarioides (17a)
H. shuangpaiensis (4)
H. sinuata (3)
H. sinuata (9)
H. yixianensis (2)
H. changhuanensis (11)
H. lichuanensis (13a)
H. lichuanensis (13b)
H. paradoxa (15)
H. warburgii (19)
C. zhejiangensis (21a)
H. warburgii (18)
C. zhejiangensis (21b)
Cardamine flexuosa
Rorippa palustris
100%
79%
100%
100%
100%
97%
78%
100%
90%
96%
100%
96%
100%
99%
100%
100%
10
distance
Y. acutangula
Y. lichuanensis
Y. rivulorum
Y. fumarioides
Y. acutangula
Y. fumarioides
Y. rupicola
Y. lichuanensis
Y. lichuanensis
Y. lichuanensis
Y. lichuanensis
Y. fumarioides
Y. fumarioides
Y. fumarioides
Y. fumarioides
nomenclature according
to Al-Shehbaz et al., 1998
trnL-phylogeny
Y. acutangula (5, 6, 23, 24)
Y. henryi (7)
Y. furcatopilosa (8, 22)
H. guangdongensis (12)
H. hunanensis (16)
H. alatipes (10)
C. zheijangensis (20)
H. rupicola (14)
Y. qianningensis (1)
H. fumarioides (17)
H. shuangpaiensis (4)
H. sinuata (3)
H. sinuata (9)
H. yixianensis (2)
H. changhuanensis (11)
H. lichuanensis (13, 25, 26)
H. paradoxa (15)
H. warburgii (19)
C. zhejiangensis (21)
H. warburgii (18)
Cardamine flexuosa
Rorippa palustris
97%
98%
87%
76%
89%
76%
10
distance

grouped together by ITS data. Accession number 12 (*H. guangdongensis*) is unrelated to these accessions based on ITS data. Similarly, *H. warburgii* (O. E. Schulz) Y. H. Zhang & H. W. Li (acc. nos. 18, 19), *Cochleariella zhejiangensis* (acc. nos. 20, 21), and *H. fumarioides* (acc. no. 17) merge into *Y. fumarioides* (Dunn) Y. Z. Zhao, as proposed by Al-Shehbaz et al. (1998). ITS sequences from *Y. fumarioides* sensu Al-Shehbaz et al. (1998) are found in three different positions among the *Hilliella/Cochleariella* clade (Fig. 2).

*trn*L DATA

The alignment of 514 bp is interspersed with seven gaps as shown in Figure 3. Sequence data were submitted to GenBank with accession numbers AF100853 through AF100881 (Table 1). The lengths of *trn*L intron sequences range from 311 bp in *Hilliella rivulorum* to 514 bp in *Cardamine flexuosa*. Of the 38 variable nucleotide positions in the alignment, 12 sites are autapomorphic. In addition, one of the seven gaps is autapomorphic. Phylogenetic analysis resulted in two MPTs with a length of 53 steps and a consistency index of 92.5% (90.0% if autapomorphies were excluded). One most parsimonious tree is shown to demonstrate relative branch length. The strict consensus tree could be generated easily by drawing branches that are indicated in Figure 2 by heavy bars with zero length. Bootstrap values are given from 1000 replicates.

Discrimination of a *Yinshania* clade from a *Cochleariella/Hilliella* clade is not as obvious with *trn*L intron data as compared to ITS sequence data (Fig. 2). In the *trn*L tree the branch setting of the *Yinshania* clade as a sister group to the *Hilliella/Cochleariella* clade is not highly supported, and bootstrap value for this branching point is less than 50% (Fig. 2). Removing additional gap characters from the matrix parsimony analysis resulted in one MPT with the *Yinshania* clade as a sister group to *H. hunanensis* (acc. no. 16), *H. guandongensis* (acc. no. 12), *C. zhejiangensis* (acc. no. 20), and *H. alatipes* var. *micrantha* (acc. no. 10). Remaining *Hilliella/Cochleariella* taxa appeared in this analysis for *trn*L data excluding gap information as a sister group to these two clades. Nonetheless, integration of *Yinshania qianningensis* Y. H. Zhang into the *Hilliella/Cochleariella* clade is significant for *trn*L data, in contrast to its segregation by ITS. Estimation of decay indices (DI) using the *trn*L intron matrix without gap information revealed a high value, DI = 3+, for the branch setting of the *Yinshania* clade. Different accessions of *C. zhejiangensis* (acc. nos. 20, 21) did not group closely together; this has also been documented for the ITS data (Figs. 2, 6). *Hilliella changhuaensis, H. guangdongensis,* and *H. lichuanensis* do not group together in the cpDNA-based tree as proposed by the morphology-based concept combining them in *H. lichuanensis* (Al-Shehbaz et al., 1998); the same discordance holds for *H. warburgii, C. zhejiangensis,* and *H. fumarioides.* Based on the *trn*L sequence data, they are not combined in one single clade that could be named as *H. fumarioides* as proposed in the revison of Al-Shehbaz et al. (1998).

ITS VERSUS TRNL INTRON DATA

Both phylogenetic trees (ITS vs. *trn*L data) from Figure 2 show some congruencies (all following arguments are also true when comparing the strict consensus trees from Fitch parsimony, which could be easily deduced by drawing branches indicated by heavy bars with zero length):

(1) *Hilliella guangdongensis* [sensu Zhang], which has been merged in *H. lichuanensis* sensu Al-Shehbaz et al. (1998), is separated from remaining *H. lichuanensis* sensu Al-Shehbaz et al. (1998), and it is more closely related to *H. hunanensis,* a taxon that was recognized by Al-Shehbaz et al. (1998).

(2) *Hilliella warburgii* and *Cochleariella zhejiangensis* are not integrated into *H. fumarioides* sensu Al-Shehbaz et al. (1998), but (3) most accessions

←

Figure 2. Comparison of the ITS-derived phylogeny with those from the *trn*L sequence data from *Yinshania, Cochleariella,* and *Hilliella,* as well as outgroups *Cardamine* and *Rorippa* from subtribe Arabideae (Brassicaceae). Accession enumeration in brackets follows Table 1. For the ITS and the *trn*L tree the nomenclature sensu Zhang (1985, 1986, 1987, 1993) has been used. In between both trees the taxonomic treatment of these taxa according to Al-Shehbaz et al. (1998) is shown. The most parsimonious tree (weighted parsimony approach) from ITS nuclear sequence data and one out of two MPTs from the *trn*L plastid sequence data is shown to demonstrate relative branch length. Weakly supported branches that collapsed in the strict consensus trees using Fitch parsimony are highlighted by a broad line. (The strict consensus trees could therefore be easily generated by drawing broad lines as zero branches.) Bootstrap values are provided from 1000 replicates and shown above branches if greater than 50%. Distance scale indicating branch lengths is given below. ITS designation (a) and (b) of accession nos. 5, 7, 8, 13, 17, and 21 indicate different ITS types from a single individual.

```
      gaps:          a     b
                1                                                                100

C. FLEXUOSA   AATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCTTGT
R. PALUSTRIS  AATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCTTGT
5-6-24-25     AATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCTGGG
7-8-23        AATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCTGGG
1-14-17       AATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCCGGT
2-3-4-11      AATTGGAT-GAGCCTTGGTATGGAAACCTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCCGGT
15            AATTGGAT-GACCC-TGGTATGGAAACCTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCCGGT
9             AATTGGAT-GAGCCTTGGTATGGAAACCTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCCGGT
13-26-27      AATTGGAT-GAGCC-TGGTATGGAAACCTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCCGGT
18-19         AATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCCGGT
21            AATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCCGGT
20            AATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCTGGG
10            AATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCTGGG
16            AATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCTGGG
12            AATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCTGGG

      gaps:                                  c
                101                                                              200

C. FLEXUOSA   TTACGCGAACAAACCTGAGTTTAGAAAGCGAGAAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGAAGTTCACTACCTTGTGTTG
R. PALUSTRIS  TTACGCAAACAAACCCGAGTTTAGAAAGCGAGAAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACCTTGTGTTG
5,6,24,25     TTACGCGAACAAACCGGAGTTTCGAAAGCGAGAAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACCTTGTGTTG
7,8,23        TTACGCGAACAAACCGGAGTTTCGAAAGCGAGAAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACCTTGTGTTG
1,14,17       TTACGCGAACAAACCAGAGTTTAGAAGGCGAGAAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACTTTGTGTTG
2,3,4,11      TTACGCGAACAAACCAGAGTTTAGAAGGCGAGAAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACTTTGTGTTG
15            TTACGCGAACAAACCAGAGTTTAGAAGGCGAGAAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACTTTGTGTTG
9             TTACGCGAACAAACCAGAGTTTAGAAGGTGAGAAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACTTTGTGTTG
13,26,27      TTACGCGAACAAACCGGAGTTTAGAAGGCGAGAAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACTTTGTGTTG
18,19         TTACGCGAACAAACCAGAGTTTAGAAAGCGAGAAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACCTTGTGTTG
21            TTACGCGAACAAACCGGAGTTTAGAAAGCGAGAAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACCTTGTGTTG
20            TTACGCGAACAAAACAGAGTTTAGAAAGC---------GGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCAATCCCTTGTGTTG
10            TTACGCGAACAAAACAGAGTTTAGAAAGC---------GGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCAATCCCTTGTGTTG
16            TTACGCGAACAAAACAGAGTTTAGAAAGC---------GGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCAATCCCTTGTGTTG
12            TTACGCGAACAAAACAGAGTTTAGAAAGC---------GGGATAGGTGCAGAGACTCAATGGACGCTGTTCTAACAAATGGAGTTCAATCCCTTGTGTTG
```

Figure 3. Alignment of the plastid *trn*L intron region from *Yinshania*, *Cochlearia*, and *Hilliella*, as well as outgroups *Cardamine flexuosa* and *Rorippa palustris*. Taxon enumeration follows Table 1. Gaps are marked alphabetically, and the corresponding gap matrix is added.

```
      gaps: d                                             e        f
            201                                                                                       300

C. FLEXUOSA  -ATAAAGGAATCCTTCGATCGAAACTTCAAATCAAAAAGGATGCAGGAGAAAAGCCTATATTGTCTAAATAAAGGTAACACAAAACGATCTCAAAAACGA
R. PALUSTRIS -ATAAAGGAATCCTTCGATCGAAACTTCAACTCAAAAAGGATGAAGGAGAAA----------GTCTGAATATAGGTAACACAAAACGATCTCAAAAATAA
5,6,24,25    -ATAAAGGAATCCTTCGATCGAAACTTCAAATCAAAAAGGATGAATGAGAAAAACCTATATTGTCTAAATATAGGTAACACAAAACGATCTCAAAAATGA
7,8,23       -ATAAAGGAATCCTTCGATCGAAACTTCAAATCAAAAAGGATGAATGAGAAAAACCTATATTGTCTAAATATAGGTAACACAAAACGATCTCAAAAATGA
1,14,17      -AT-------------------------------------------------------------------------------------------------
2,3,4,11     -AT-------------------------------------------------------------------------------------------------
15           -AT-------------------------------------------------------------------------------------------------
9            -AT-------------------------------------------------------------------------------------------------
13,26,27     -AT-------------------------------------------------------------------------------------------------
18,19        -AT-------------------------------------------------------------------------------------------------
21           -AT-------------------------------------------------------------------------------------------------
20           AAT-------------------------------------------------------------------------------------------------
10           AAT-------------------------------------------------------------------------------------------------
16           AAT-------------------------------------------------------------------------------------------------
12           AAT-------------------------------------------------------------------------------------------------


      gaps:
            301                                                                                       400

C. FLEXUOSA  CGACCTGAATCTCGATTTCTATTTTTTTATAAACAAAATAGAAATGTTGTGAATCAATTCGAAGTTTAAGACAAAATCAAATATTCATTGATCAAATAAT
R. PALUSTRIS CGACCTGAATCTCGATTTCTATTTTTTTATAAACAAAATAGAAATGTTGTGAATCAATTCGAAGTTTAAGAGAAAATCAAATATTCATTGATCAAATGAT
5,6,24,25    CGACCTGACTCTCGATTTCTATTTTTTTCTAAACAAAATAGAAATGTTGTGAATCAATTCGAAGTTTAAGAAAAAAATCGAATATTCATTGATCAAATGAT
7,8,23       CGACCTGACTCTCGATTTCTATTTTTTTCTAAACAAAATAGAAATGTTGTGAATCAATTCGAAGTTTAAGAAAAAAATCGAATATTCATTGATCAAATGAT
1,14,17      --------------------------------------------------------------------------------------------CAAATGAT
2,3,4,11     --------------------------------------------------------------------------------------------CAAATGAT
15           --------------------------------------------------------------------------------------------CAAATGAT
9            --------------------------------------------------------------------------------------------CAAATGAT
13,26,27     --------------------------------------------------------------------------------------------CAAATGAT
18,19        --------------------------------------------------------------------------------------------CAAATGAT
21           --------------------------------------------------------------------------------------------CAAATGAT
20           --------------------------------------------------------------------------------------------CAAACGAT
10           --------------------------------------------------------------------------------------------CAAACGAT
16           --------------------------------------------------------------------------------------------CAAACGAT
12           --------------------------------------------------------------------------------------------CAAACGAT
```

Figure 3. Continued.

```
        gaps:        g
              401                                                                                                  500

C. FLEXUOSA   TCACTTCATACATAGTCTGATAGATCCTTGGTGGAACTTATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTGAATACTGACAACAATGAA
R. PALUSTRIS  TCACTT----CATAGTCTGATAGATCCTTGGTGGAACTTATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTGACAACAATGAA
5,6,24,25     TTACTT----CATAGTCTGATAGATCCTTGGTGGAACTTATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTGACAACAATGAA
7,8,23        TTACTT----CATAGTCTGATAGATCCTTGGTGGAACTTATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTGACAACAATGAA
1,14,17       TCACTT----CATAGTCTGATAGATCCTTGGTGGAACTTATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTGACAACAATGAA
2,3,4,11      TCACTT----CATAGTCTGATAGATCCTTGGTGGAACTTATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTGACAACAATGAA
15            TCACTT----CATAGTCTGATAGATCCTTGGTGGAACTTATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTGACAACAATGAA
9             TCACTT----CATAGTCTGATAGATCCTTGGTGGAACTTATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTGACAACAATGAA
13,26,27      TCACTT----CATAGTCTGATAGATCCTTGGTGGAACTTATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTGACAACAATGAA
18,19         TCACTT----GATAGTCTGATAAATCCTTGGTGAAACTTATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTGACAACAATGAA
21            TCACTT----GATAGTCTGATAAATCCTTGGTGAAACTTATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTGACAACAATGAA
20            TCACTT----CATAGTCTGATAGATCCTTGGTGGAACTTATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTGACAACAATGAA
10            TCACTT----CATAGTCTGATAGATCCTTGGTGGAACTTATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTGACAACAATGAA
16            TCACTT----CATAGTCTGATAGATCCTTGGTGGAACTTATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTGACAACAATGAA
12            TCACTT----AATAGTCTGATAGATCCTTGGTGGAACTTATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTGACAACAATGAA


        gaps:                      abcdefg
              501         515

C. FLEXUOSA   ATTTATAGTAAGATG     1110111
R. PALUSTRIS  ATTTATAGTAAGATG     1110100
5,6,24,25     ATTTATAGTAAGATG     1110110
7,8,23        ATTTATAGTAAGATG     1110110
1,14,17       ATTTATAGTAAGATG     1110010
2,3,4,11      ATTTATAGTAAGATG     0110010
15            ATTTATAGTAAGATG     0010010
9             ATTTATAGTAAGATG     0110010
13,26,27      ATTTATAGTAAGATG     0010010
18,19         ATTTATAGTAAGATG     1110010
21            ATTTATAGTAAGATG     1110010
20            ATTTATAGTAAGATG     1101010
10            ATTTATAGTAAGATG     1101010
16            ATTTATAGTAAGATG     1101010
12            ATTTATAGTAAGATG     1101010
```

Figure 3. Continued.

of *H. warburgii* and *C. zhejiangensis* formed one single cluster with no separation of *H. warburgii* versus *C. zhejiangensis*. This is in agreement with previous concepts combining both taxa, *C. zhejiangensis* and *H. warburgii*, in one single taxon. These congruent ITS and *trn*L intron findings demonstrate that both *Y. lichuanensis* and *Y. fumarioides* species complexes, treated as well-defined taxa according to the morphological revision of Al-Shehbaz et al. (1998), do not form monophyletic groups by molecular evidences.

However, some incongruencies among the two molecular data sets could be detected: (1) *Yinshania qianningensis* grouped either inside the *Hilliella/Cochleariella* clade (*trn*L intron data) or into *Yinshania* s. str. (ITS data); (2) *H. rupicola* (D. C. Zhang & J. Z. Shao) Y. H. Zhang also clustered into two different subgroups within *Hilliella/Cochleariella*.

TRIBAL RELATIONSHIPS

The alignment of ITS1–5.8SrDNA-ITS2 is 644bp in length (Fig. 4). Within the ITS1 region (bp 1–301) there are 143 variable nucleotide positions (including 62 autapomorphies). The ITS2 region (position 456–644) contains 85 variable nucleotide positions (including 57 autapomorphies). The 5.8S rDNA gene, located between both spacer regions (bp 302–455), contains 11 variable nucleotide positions (including 6 autapomorphies). Because of an ambiguous alignment, nucleotide positions 107–153 and 460–484 were removed from the original data matrix (Fig. 4), resulting in a final data matrix of 412 bp.

Fitch parsimony analysis resulted in one MPT with a length of 288 steps and a consistency index of 79.5% (66.5% if autapomorphies are excluded). The phylogenetic tree (Fig. 5) showed closer relationships of *Yinshania* to taxa from tribe Arabideae sensu Janchen (1942) (*Arabidopsis* (DC.) Heynh., *Barbarea* R. Br., and *Cardamine* L. were used to represent tribe Arabideae). However, *Cochlearia* (including species loosely affined to the *Yinshania* complex) and *Capsella rubella* were placed by Janchen in the tribe Lepidieae, where they had been put by Hayek (1911) and Schulz (1936). Our ITS data do not support this latter placement. A molecular analysis of *Arabidopsis*, *Arabis* L., and their relatives shows a close relationship of *Capsella rubella* to *Arabidopsis thaliana* (Koch et al., 1999b). Also demonstrated is the polyphyly of *Arabis* and *Arabidopsis*, indicating that tribal structures within Brassicaceae are highly artificial. The ITS phylogeny does suggest that Chinese *Yinshania* and related taxa are closer to *Cardamine* and *Barbarea* from tribe Arabideae than to genera like *Thlaspi* or *Cochlearia* s. str. of the tribe Lepidieae.

CYTOLOGY

Little is known about the cytology of *Yinshania*. Zhang (1995, and pers. comm.) counted $2n = 12$ for *Y. qianningensis* Y. H. Zhang [= *Y. acutangula* sensu Al-Shehbaz et al., 1998], *Y. henryi* (Oliv.) Y. H. Zhang, and *Y. furcatopilosa* (K. C. Kuan) Y. H. Zhang, $2n = 42$ for *Hilliella yixianensis* Y. H. Zhang, *H. paradoxa* (Hance) Y. H. Zhang & H. W. Li, and *H. changhuaensis* [= *Y. lichuanensis* sensu Al-Shehbaz et al., 1998], and $2n = 44$ for *H. shuangpaiensis* Z. Y. Li [= *Y. rupicola* sensu Al-Shehbaz et al., 1998]. These data correspond to the ITS-derived phylogeny, in which the diploid *Y. qianningensis*, *Y. henryi*, and *Y. furcatopilosa*, together with *Y. acutangula*, are separated from the polyploid *Hilliella*. Within polyploid *Hilliella*, the *Hilliella* taxa with $2n = 42$ are combined. *Hilliella shuangpaiensis* (represented in this study by acc. no. 4, Fig. 2) with $2n = 44$ did not group closely to the known $2n = 42$ taxa (represented in this study by acc. nos. 2, 11, and 15, Fig. 2). However, any conclusions based on cytology must be only preliminary. It remains possible that the *Hilliella/Cochleariella* group could also be represented by polyploid taxa with $2n = 42$ and 44. Base chromosome number for this hexaploid group is $x = 7$, instead of $x = 6$ as in the *Yinshania* group. A few taxa within the *Hilliella/Cochleariella* clade may be aneuploids ($2n = 44$) that derived from $2n = 42$.

DISTRIBUTION

Some interesting features emerge when topologies of the phylogenetic trees are compared to the geographic distribution of *Yinshania* s.l. Geographic distribution of ITS sequence types from taxa of the four main clades within *Hilliella/Cochleariella* do not follow their phylogenetic relationships (Fig. 7), and they are randomly mixed in Southeast China in the provinces of Guangdong, Jiangxi, Zhejang, Anhui, Hubei, and eastern Sichuan. However, they are separated geographically and phylogenetically from the *Yinshania* clade from Hunan, Xizang, and Sichuan. Taxa from the *Yinshania* clade extend the distribution to the southwest. A mixed distribution of DNA types also holds for the *trn*L data. No geographic structuring of plastome types could be observed among taxa from the *Hilliella/Cochleariella* clade (Fig. 8). Based on *trn*L intron data *Y. qianningensis* (acc. no. 1) from Sichuan integrates into the *Hilliella/Cochleariella* clade. Geographically,

```
             ITS1
             1                                                                                81
Arabidopsis  TCGATACCTGTCC-AAAACAGAACGACCCGCGAACCAA-AGATCACCA-CTCTCGGTGG-GCCGGTTTCTTAGCC-GATTC
Cardamine    TCGTATCCTGCCC-AAAAAAGACCGAACCGCGGACCAA-AGATCATCAACTCT-GGTGA-GCGGGTTTCATAACTTGAGAT
Cochlearia   TCGAAACCTGTTC-AAAACAGTACGACCCGAGAACAAC-TGATCATCA-CTCTCGGTAG-GCCGGTTTCTTAACA-GTTAT
Barbarea     TCGATACCTGTCC-AAAACAGAACGACCCGCGAACCAA-ATATCATCA-CTCTCGGTGG-GCCGGTTTCTTAGCT-GAGAT
Capsella     TCGATACCTGTCC-AAAACAGAACGACCCGAGAACCAA-AGATCACCA-CTCGCGGTAAAGCCGATTTCGTAACA-GATTT
Sinapis      TCGT-ATCTGG----AAACAGAACAACCCGAGAACAATGAAAACATCA-CTCTCGGTGG-GCCGGTTTCTTTGCT-GATTC
Brassica     TCGT-ACCCGGG---AAACAGAACGACCCGAGAACGTTGAAA-CATCA-CTCTCGGTGG-GCCGGTATCTTAGCT-GATTT
Yinshania    TCGATACCTGTCCAAAAACAGAACGACCCGCGAACCAA-AGATCATCA-CTCGCGGTAG-GCCGGTTTCTTAGCC-GATCC
Thlaspi      TCGTAACCTGTT-AAAAACAGAACGACCCGAGAACAAT-CGATCATCA-CTCTCGGTGG-GCCAGTTTCTTAAAT-GATCT

             82                        ΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔ         162
             CTTGCCC--GCCGGATCCGTGGTTTCGCGTATCGGCATGGTCGGGAGCTTTTATCTCGGTCTTGTC-----GTGCGCGTTG
             CGTGCCT--GCCGAATCCGTGGTTTCGCCGAACAATCCTTACCGGGAGCTTAATCTCTGTTTGGATT----GTGCGCGTTG
             CGTGCCT--ACCGATTCCGTGGTTTT-CATGTTTGGCTTCGTCAGTATTCATTACATGGCGAAGTC-----ATGCATGTTT
             CGTGTCTCTGCCGAATCCGTGGTTTCGTGAACAATCCTTACCGGGAGCTCTCTCTCTGTTTGGGTT-----GTGCGCGTAG
             CGTGCCT--GCCGAATCCGTGGTTTCCCGTACCTTCCCGGTCGAGAGTTTTCTCTCGGTCTGGTC------GTGCGCGTTG
             TGTGCCT--GCCGATTCCGTGGTT-ATGCGTTAAGTTCCCAGCCAGTACTTCAGTCTTGGTTGGGTC----TTGCGCATTG
             CGTGCCT--ACCGATTCCGTGGTT-ATGCGTTCGTCACCGGCCTAGNTTTCGGTTGGATT-----------GTACGCATAG
             CGTGCCT--GCCGAGTCCGTGGTTTCGCGTATTGTCCCGGGCGAGAGCTCAATCTCGGTCGTGTC------GTGCGCGTAG
             TGTGCCT--GCCGATTCCGTGGTTTCGCGTACGATTCTCATCAAGGTATATATATATATCTTGGTTTGATCATGCGTGTAG

             163                                                                             243
             CTTCCGGATATCACAAAACCCCGGCACGAAAAGTGTCAAGGAACATGCAAACGAACGGCTG--GCATTCGCCTCCCCGGAG
             CTTCCGGATATCACAAAACCACGGCACGAAAAGTGTCAAGGAACATGCAATTGAACAGACA--GCCTTCGCCTCCCCGGAG
             CCTCCGGATTTCACCAAACCACGGCACGAAAAGTGTCAAGGAACATGCAACTAAACAGCTT--GCATTCGCCGCACCAGAG
             CTTCCGGATATCACAAAACCACGGCACGAAAAGTGTCAAGGAACATGCATATGAACAGCAA--GCCTTCGCCTCTCCAGAG
             CTTCCGGATATCACAAAACCCCGGCACGAAAAGTGTCAAGGAACATGCAACCGAACGGCTATAGCATTCGCTCCCCCGGAG
             CTTCCGGATATCACCAAACCCCGGCACGAAAAGTGTCAAGGAACATTCAACTAGGTAGCCT--GCTTTCGCCAACCCGGAG
             CTTCCGGATATCACCAAACCCCGGCACGAAAAGTGTCAAGGAACATTCAACTAAACAGCCT--NNTTTCGCCAACCCGGAG
             CTTCTGGATATCACCAAACCACGGCACGAAAAGTGTCAAGGAACATGCAACAGAACGGCCA--GCCTTCGCCTCCCCGGAT
             CTTCCGGTTATCACAAAACCCCGGCACGAAAAGTGTCAAGGAACATGCAACTAAACAGCCA--GCGTTTGCCTTCCCGGAG
```

Figure 4. Alignment of ITS1, 5.8S rDNA, and ITS2 regions from different crucifers. Characters excluded from data analysis are marked (Δ). For sequence accession data refer to the materials and methods section.

5.8SrDNA

```
244                                                                        324
ACGGAGTGTGG-GCGGATGCTGT-GCTGCGAACTGAAGTCTAAAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCG
ACGGTGTGTGT-GCGGATCCTGC-GCTGCGATCTAAAGTCTAAAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCG
ATGGTTTGTGT-GCGAATG-TCTCGATGC-ATTTAAAGTCTAAAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCG
ACGGTGTGCGT-GCGTTTGGTGA-GCTGCGATCAAAAGTCTAAAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCG
ACGGTGTG-GTCGCGGATGCT-TAGCTGCGATATAAAGTCTAAAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCG
ACGGTGTTTGT-GCGGAAGCTGT-GCTCCAATGTAAAGTCTAAAACGACTCTCGGCAACGGATATCTTGGCTCTCGCATCG
ACGGTGTTTGT-TCGGAAGCAGT-GCTGCAATGTAAAGTCTAAAACGACTCTCGGCAACGGATATCTCGGCTCTCACATCG
ACGGTGTGAGT-GCGGATGTTGT-GCTGCGATCTAAAGTCTAAAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCG
ACGGTGTTTGC-GTGAACGCTTT-GCTGCAATTTAAAGTCTAAAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCG

325                                                                        405
ATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGC
ATGAAGAAGCTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAAGCCAAGTTGCGC
ATGAAGAAGCTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAAGCCAAGTTGCGC
ATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGC
ATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAGCCATCGAGTCTTTGAACGCAAGTTGCGC
ATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTGCAAGATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGC
ATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGC
ATGAAGAAGCTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAAGCCAAGTTGCGC
ATGAAGAAGCTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAAGCCAAGTTGCGC

                                                    ITS2
406                                                 ΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔ 486
CCCAAGCCTTCTGGCCGAGGGCACGTCTGCCTGGGTGTCACAAATCGTCGTCCCTCA-CCATCC-T-TTGCTGA-TGCGGG
CCCAAGCCTTCTGGCCGAGGGCACGTCTGCCTGGGCGTCACAAATCGTCGTCCC-TC-TCATCC-TCTT-AGGACG-TGGG
CCCAAGCCTTCTGGCCGAGGGCACGTCTGCCTGGGCGTCACAAATCGTCGTCC-ATC-A-ATCC-TATA-AGGATTTT-GG
CCCAAGCCTTCTGGCCGAGGGCACGTCTGCCTGGGTGTCACAAACCGTCGTCCCTC--TCATTCTTCT-C-GGA-TATGGG
CCCAAGCCTTCTGGCCGAGGGCACGTCTGCCTGGGTGTCACAAATCGTCGTCCC-CCCTCATCCTTCA--AGGATT-CGGG
CCCAAGCCTTCTGGCCGAGGGCACGTCTGCCTGGGTGTCACAAATCGTCGTCCC-CC--CATCC-TCTCGAGGA-TATGGG
CCCAAGCCTTCTGGCCGAGGGCACGTCTGCCTGGGTGTCACAAATCGTCGTCCN-CC--AATCC-TCTCGAGGA-TATCGG
CCCAAGCCTTCTGGCCGAGGGCACGTCTGCCTGGGCGTCACAAATCGTCGTCCC-CT-TCATCC-TTG-CG-GA-TACGGG
CCCAAGCCTTCTGGCCGAGGGCACGTCTGCCTGGGCGTCACAAATCGTCGTCCC-C---CATCC-TCTTAAGGA-TACGGG
```

Figure 4. Continued.

```
487                                                                           567
ACGGAAGCTGGTCTCCCGTGTGTTACCGCACGCGGTTGGCCTAAATCCGAGCC-AAGGACG-CCTGGAGCGTACCGACATG
ACGGAAGCTGGTCTCCCGTGTGTTACCGCACGCGGTTGGCCCAAATCCGAGC-TAAGGACG-CCGAGAGCGTCACGACATG
ACGGAAGCTGGTCTCCCGTGTGTTACCGCACGCGGTTGGCCAAAATCCGAGCCTA-GGACGCCCAG-AGCGTCTCGACATG
ACGGAAGCTGGTCTCCCGTGTGTTACCGCACGCGGTTGGCCAAAATCCGAGC-TAAGGGCG-CCTAGAGCGTCACGACATG
ACGGAAGCTGGTCTCCCGTGTGTTACCGCACGCGGTTGATCGAAATC-GAGCC-AAGGATG-CCTTGAGCGTCCCGACATG
ACGGAAGCTGGTCTCCCGTGTGTTACCGCACGCGGTTGGCCAAAATCCGAGC-TAAGGACGTTTTGGAGCGTCTCGACATG
ACGGAAGCTGGTCTCCCGTGTGTTACCGCACGCGGTTGGCCAAAATCCGAGC-TAAGGATG-CCAGGAGCGTCTTGACATG
ACGG-AGCTGGTCTCCCGTGTGTTACCGCACGCCGTCGGGCAAAATCCGAGCAAAAGGACC-CG-GGAGCGTCCCGACATG
ACGGAAGCTGGTCTCCCGTGTTTTACCGAATGCGGT-GGCCAAAATCTGAGC-TAAGGACG-CCAGGAGTGTCTCGACATG

568                                                                       644
CGGTGGTGAACTTGATCCATTA-CA-TTTTATCGGTCGCTCTTGTCCGGAAGCTGTAGATGACCCAAAGTCCATATA
CGGTGGTGAACTAAAGCCTGT-TCG-TATCGTCGGTCGTTCTTGTCTATAAGCTCTCGATGACCCAAAATCCTCAAA
CGGTGGTGAAAT-AAGCCTCT-TCA-TAACGTCGTGCGCTCTTGTCCAAAAGCTCCCGATGACCCAATGTCTTCTAT
CGGTGGTGAACTAAAGCCCCT-TCG-TATTGTCGGTCGTTCTTGTCCGGAAACTCTCGATGACCCAAAGTCTTTAAA
CGGTGGTGAACTCGTTCAACTCTCCCTATCGTCGGTCGCTCTTGTCCGGAAGCTCTAGATGACCCAAAGTCTTCAAT
CGGTGGTGAATTGTAACCTCG-TCA-TATTGTCGGTCGTTCCGGTTCAAAAGCTCTTGATGACCCAAAGTCCTCAAC
CGGTGGTGAATTCAATTCTCG-TCA-AATCGTCAGTCGTTTCGGTCCGAAAGCTCTTGATGACCCAAAGTCCTCAAC
CGGTGGTGTGTGCAAGCCTCTATAA-TATCGTCGGCCGCTCTTGTCCG-AAGCTCTA-AT-ACCCAAAGTT-TCAAG
CGGTGGTGAATTCAAGCCTCTTTAG-T-TTGTCGGCCGCTCTTGTCTGGAAGCTCTTGATGACCCAAAGTCCTCAAC
```

Figure 4. Continued.

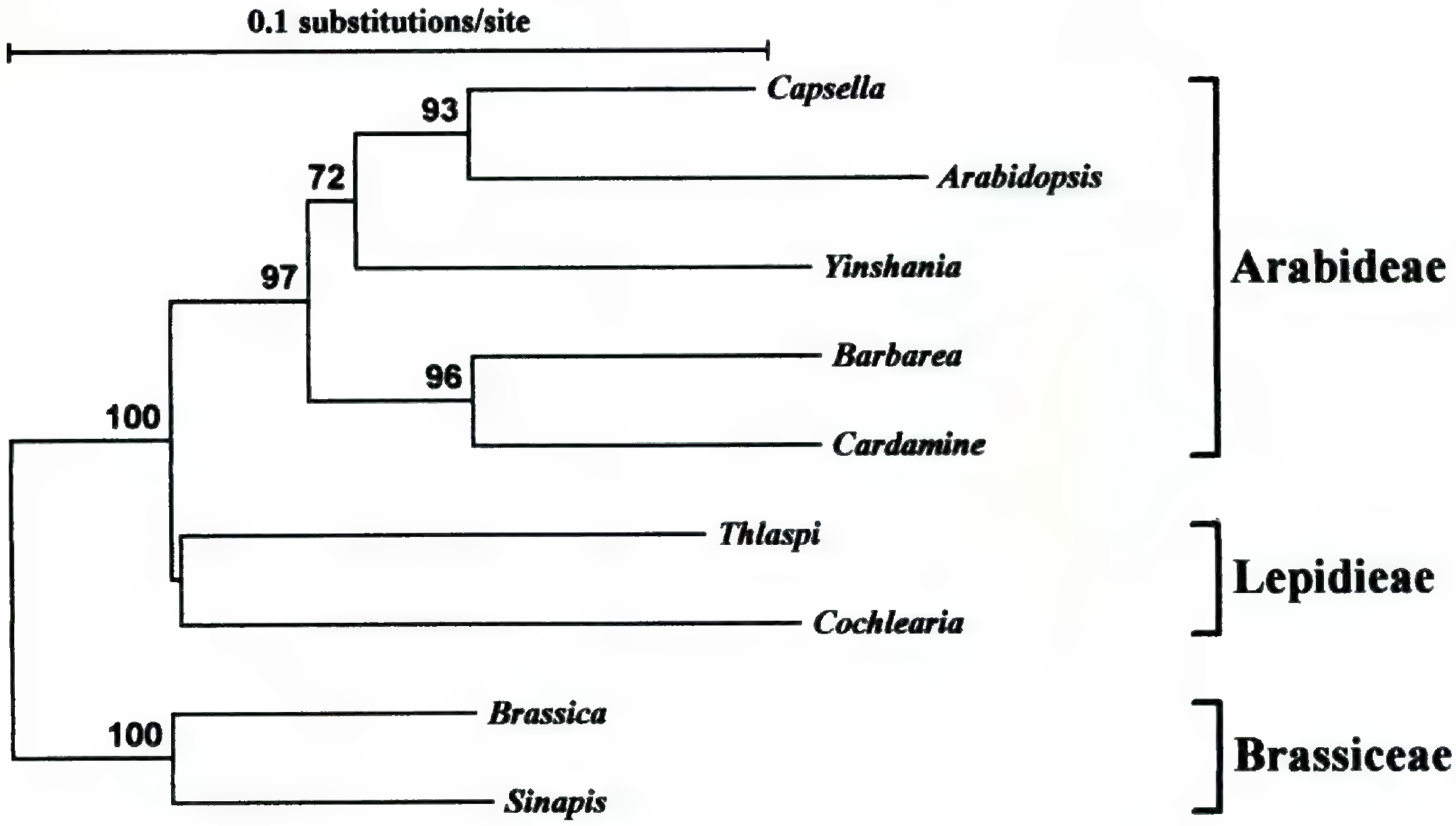


Figure 5. Phylogenetic relationships of cruciferous taxa from different tribes. Genetic distances were calculated using the program TREECON (van de Peer & de Wachter, 1994) under the Kimura model (Kimura, 1980). Gaps were not taken into account, and the neighbor joining algorithm was used to calculate genetic distances. The robustness of the tree was tested by 1000 bootstrap replicates.

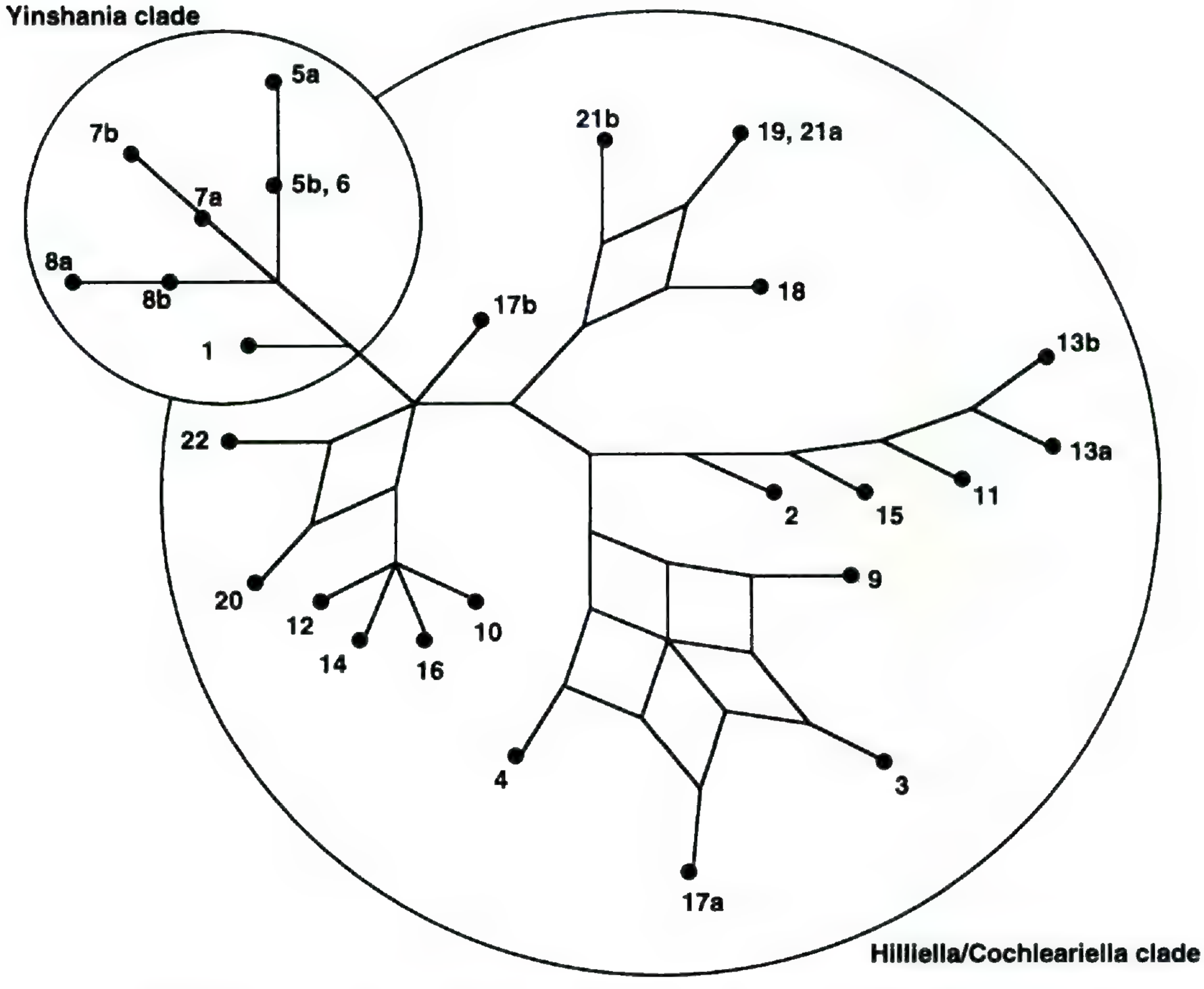


Figure 6. Split Decomposition of the ITS sequence data set analyzing the ingroup taxa. The DRAW-EQUAL-EDGES option was used to draw the network, and thus distances are not drawn to scale.

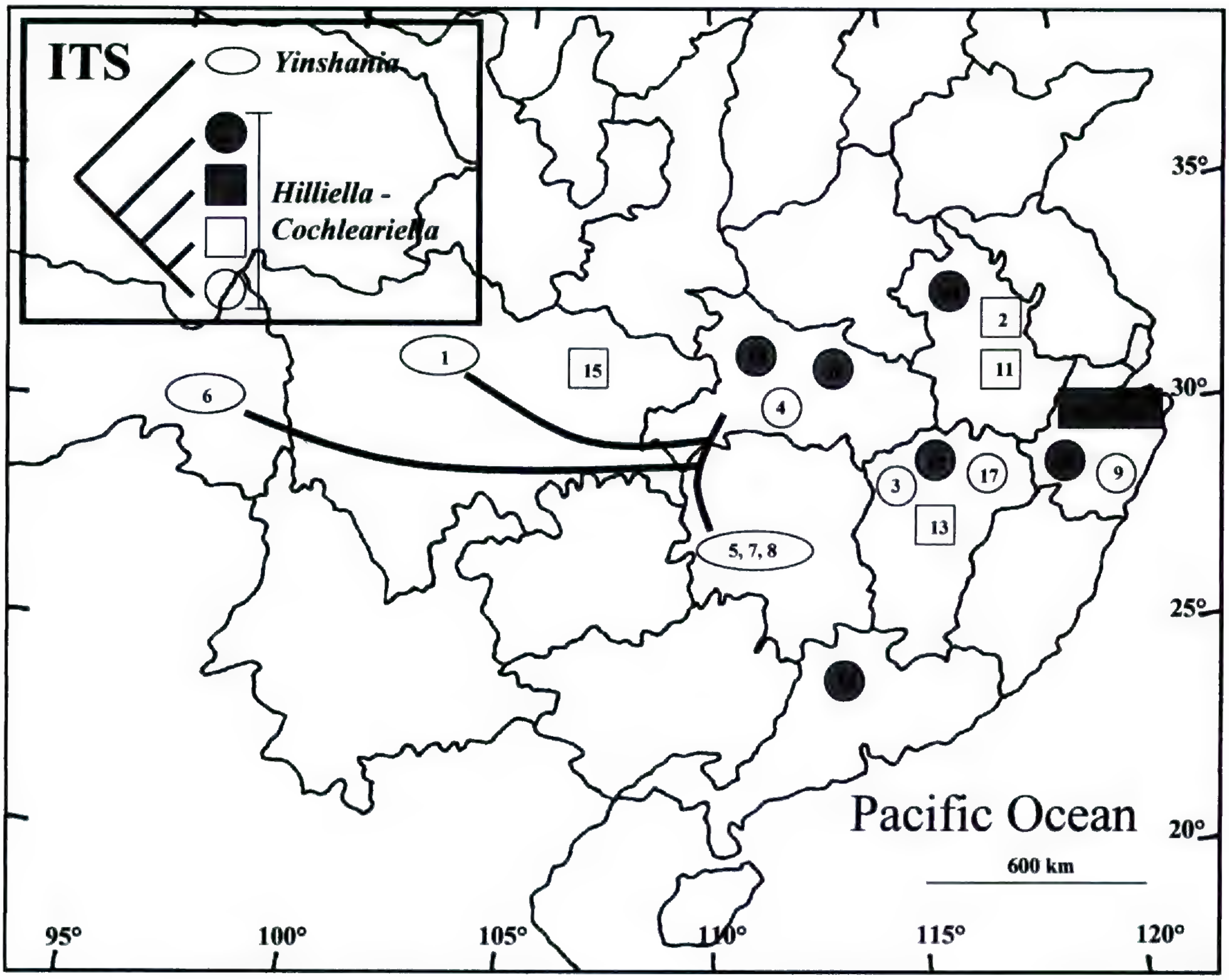


Figure 7. Distribution of ITS types among *Yinshania* and *Hilliella/Cochleariella* accessions under study in China. Taxon enumeration follows Table 1. The *Hilliella-Cochleariella* clade is separated into four ITS types indicated by circles and boxes (gray or white) well suppported by high bootstrap values in Figure 2. Accessions marked by open circles and boxes are two well-separated subgroups, which are combined to a single group in Figure 8 (plastid *trn*L data). Phylogenetic relationships are shown schematically in the upper left box and follow Figure 2. For the *Yinshania* clade phylogenetic relationships are shown to demonstrate the position of *Yinshania qianningensis* [*Y. acutangula* sensu Al-Shehbaz] (acc. no. 1) (refer to Fig. 2).

this accession lies close to a similar plastome type (acc. no. 15) from the *Hilliella/Cochleariella* clade (Fig. 8). Upon comparison of the ITS and *trn*L intron-derived phylogenies, taxa that showed different positions in the phylogenetic analysis (e.g., *H. rupicola*, acc. no. 14; *H. fumarioides*, acc. no. 17: one additional ITS sequence copy) were located at the center of the *Hilliella/Cochleariella* distributional area. We interpret these results as a first biogeographical documentation of reticulation within polyploids. *Hilliella fumarioides* (acc. no. 17) possessed a *trn*L intron type similar to samples from surrounding areas. The two ITS types found in that particular individual were also present in adjacent regions and from different species. From *Hilliella rupicola* (acc. no. 14) we could isolate an ITS DNA type, which is also present in adjacent regions in accession numbers 10, 16, 17, and 20. *trn*L intron DNA type from accession number 14 also corresponds to *trn*L intron DNA types from 2, 3, 11, 13, 17, and 20.

Morphological Variation and Taxonomic Considerations

As in numerous other cases in the Brassicaceae, morphological differentiation among and within so-called genera and taxa of the *Yinshania* s.l. complex does not provide ample characters to draw sharp and uncontroversial generic boundaries. Flower morphology in the entire complex is of no predictive diagnostic value, even for the separation of species (Al-Shehbaz et al., 1998). Only leaves and fruits offer characters useful for the separation of species, and all taxa appear to be not well defined. As shown by Al-Shehbaz et al. (1998), seed sculpture and number per locule, cotyledonary position, development of the fruit septum, type (if any)

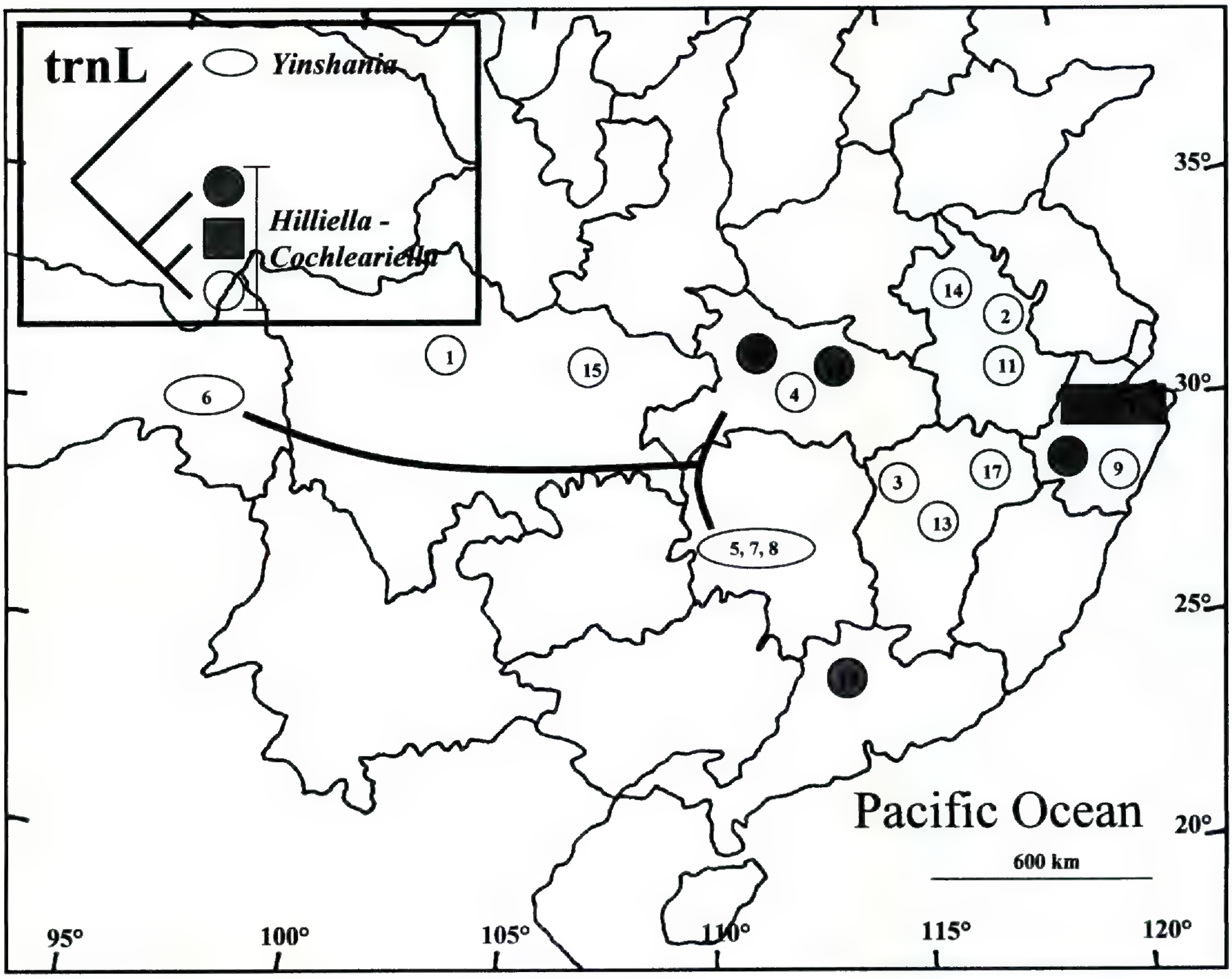


Figure 8. Distribution of *trn*L intron types among *Yinshania* and *Hilliella/Cochleariella* accessions under study in China. Taxon enumeration follows Table 1. The *Hilliella-Cochleariella* clade is separated into three plastid *trn*L types indicated by circles and boxes (gray or white) well suppported by high bootstrap values in Figure 2. Phylogenetic relationships are shown schematically in the upper left box and follow Figure 2. For the *Yinshania* clade phylogenetic relationships are shown to demonstrate the position of *Yinshania qianningensis* [*Y. acutangula* sensu Al-Shehbaz] (acc. no. 1) (refer to Fig. 2).

of fruit compression, fruit shape, development of papillae on the fruit valve, and trichome type, all are unreliable in dividing the complex into the genera *Yinshania, Hilliella,* and *Cochleariella.* In fact, no single character or set of characters can be relied upon to subdivide the complex into generic or infrageneric taxa. The recognition of a single genus is, therefore, taxonomically expedient, as there is no single character setting one clade apart from another. Even pustules on the testa surface of the valves, which were assumed to be a good character to separate *Yinshania* (pustules present) from *Hilliella* (Zhang & Xu, 1990), appear not to be congruent, failing to provide a good argument to split *Yinshania* into several genera (Al-Shehbaz et al., 1998).

Morphological Cluster Analysis

A cluster analysis of morphological and ecological characters has been performed by Zhang and Xu (1990). This analysis considered distribution, altitude, and habit, but also morphological characters describing hairs, inflorescences, flower details, silicles, seeds, and cotyledons. They concluded that minute pustules on the testa surface of the valves were a good character to separate *Yinshania* from *Hilliella.* Comparison of our molecular phylogenies with this cluster analysis based on 32 morphological and 3 ecological characters demonstrated the lack of congruency between morphological and molecular evolution. This cluster analysis separated *Yinshania* sensu Ma and Zhao (1979) from *Hilliella* sensu Zhang and Li (Zhang, 1986) (a similar resolution to the ITS data). Taxa such as *H. paradoxa, H. lichuanensis,* and *H. changhuanensis* are closely related to each other (as confirmed by molecular data by acc. nos. 11, 13, and 15 herein). However, this morphological cluster analysis also grouped *H. guangdongensis* (acc. no. 12 herein)

into this group (*Y. lichuanensis* clade, Fig. 2). For both ITS and *trn*L intron sequence, *H. guangdongensis* is very divergent from others grouping with the *Y. lichuanensis* clade (Fig. 2). *Hilliella alatipes* var. *micrantha* and *H. sinuata* are separated with both molecular markers (Fig. 2), but are combined as a well-supported group in morphological cluster analysis (Zhang & Xu, 1990). In summary, within *Hilliella* there is little morphological agreement with our molecular data: (1) *H. paradoxa, H. changhuaensis,* and *H. lichuanensis* are closely related to each other (as *Y. lichuanensis* clade, Fig. 2); (2) *H. hunanensis* and *H. rupicola* grouped together on the ITS tree (but not based on *trn*L data, Fig. 2). No further correlation could be observed. None of the three data sets (ITS, *trn*L intron, morphology) is powerful enough to elucidate phylogenetic signal for the whole species complex. However, significant correlations could be observed when cpDNA- and nrDNA-derived phylogenies were compared, dividing the *Hilliella/Cochleariella* clade into several subgroups (Fig. 2) clearly separating *Yinshania* relatives from *Hilliella/Cochleariella* with the sole exception of *Y. qianningensis.*

Hybridization, Introgression, Chloroplast Capture, and Concerted Evolution

The phylogeny based on plastid *trn*L intron sequence data reflects the maternal lineages because plastids are inherited maternally in most angiosperms, including the Brassicaceae (Harris & Ingram, 1991; Reboud & Zeyl, 1994). Introgression of a chloroplast type characteristic for the *Hilliella/Cochleariella* clade into *Yinshania qianningensis* demonstrates possible gene flow between both groups. Because of the highly polyploid genomes of *H. yixianensis, H. paradoxa, H. changhuaensis,* and *H. shuangpaiensis* (represented by acc. nos. 2, 15, 11, and 4, respectively, in Fig. 2) with multiple rDNA loci, and the assumed hybridization within the *Hilliella/Cochleariella* clade and even with the *Yinshania* clade, there is a high probability of concerted ITS sequence evolution. In principle, there are three different ways that two different ITS copies evolve within a single individual: (1) unidirectional concerted evolution leads to the loss of one copy and fixation of the second (detected in *H. rupicola* acc. no. 14, herein; and in *Gossypium,* Wendel et al., 1995a); (2) both ITS copies are still present, which might be mostly the case in young hybridogenous taxa (detected in *H. fumarioides* acc. no. 17, and *C. zhejiangensis* acc. no. 21, Fig. 2; in *Krigia,* Kim & Jansen, 1994; in *Arabidopsis,* O'Kane et al., 1996); and (3) concerted evolution leads to a new ITS type that represents a mixture of the two original ITS sequences (in *Gossypium,* Wendel et al., 1995b; in *Microseris,* van Houten et al., 1993; in *Microthlaspi,* Mummenhoff et al., 1997). The third type of concerted evolution might have happened in *H. sinuata* (acc. no. 3). This accession showed a plastome type more similar to *H. shuangpaiensis* (acc. no. 4). However, ITS sequence types from putative parental ITS sequence types from *H. sinuata* (acc. no. 9) and *H. shuangpaiensis* (acc. no. 4) exhibit some additive features found in *H. sinuata* (acc. no. 3). Comparing these three sequences, there are 39 variable nucleotide positions (21 in ITS1 region, and 18 in ITS2 region). Within the ITS1 region, 17 out of 21 variable nucleotide positions (81%) are identical among the two *H. sinuata* accessions (nos. 3, 9); *H. shuangpaiensis* shared only three mutations with *H. sinuata* (acc. no. 9) and one mutation with *H. sinuata* (acc. no. 3). Within the ITS2 region both *H. sinuata* accessions have only one nucleotide position out of 18 variable nucleotide sites invariant, but *H. shuangpaiensis* (acc. no. 4) shared 15 positions (83%) with *H. sinuata* (acc. no. 3) and two positions with *H. sinuata* (acc. no. 9). These findings indicate that ITS type of *H. sinuata* (acc. no. 3) consists of a mixture of ITS types from relatives of *H. shuangpaiensis* (mostly ITS2 region) and *H. sinuata* (ITS1 region). The overall sequence divergence between *H. shuangpaiensis* (acc. no. 4) and *H. sinuata* (acc. no. 9) is 7.8%.

Concerted evolution of ITS DNA loci has been shown several times to occur in the Brassicaceae (O'Kane et al., 1996; Mummenhoff et al., 1997; Koch et al., 1998b; Franzke et al., 1998) and other families (Wendel et al., 1995a, b; Buckler et al., 1997). Sequence divergence values of ITS types from putative parents, giving rise to hybrids in which concerted evolution has been observed, ranged from 3.1% (*Microthlaspi natolicum* vs. *M. perfoliatum,* Mummenhoff et al., 1997), 5.0% (*Cardamine amara* vs. *C. rivularis* auct., Franzke et al., 1998), to 6% (*Arabidopsis thaliana* vs. *A. arenosa,* O'Kane et al., 1996) comparable to a value of 7.8% found among *H. shuangpaiensis* versus *H. sinuata.*

We conducted a split decomposition analysis to visualize conflicting phylogenetic signal indicating concerted evolution in groups among the *Hilliella/Cochleariella* clade (Fig. 6). This analysis clearly indicates hybridization with subsequent concerted evolution of ITS regions in *H. sinuata* (acc. no. 3). Concerted evolution of ITS sequences greatly influences any interpretation of the ITS phylogeny. Since diploid members of the Brassicaceae such as *Arabidopsis thaliana* typically show 2 NOR loci,

one could assume that in hexaploid *Hilliella/Cochleariella* taxa at least 6 major NOR loci are present. Therefore, sequencing of two individual ITS clones is not a sufficient survey to find all putative ITS types from a single individual. This undersampling leads to an underestimation of ITS type variation as well as the degree of hybridization and concerted evolution.

We found evidence suggesting concerted evolution and described three examples (*H. fumarioides* acc. no. 17, *H. rupicola* acc. no. 14, *H. sinuata* acc. no. 3) of possible hybridization and subsequent concerted evolution of ITS sequence. The overall amount of sequence divergence between taxa in this study demonstrates a relatively high age for the different lineages. In fact, sequence divergence values are much higher when compared to those obtained in infrageneric studies of closely related species of other Brassicaceae (e.g., *Cochlearia* s. str. < 1.75%, Koch et al. (1999a); *Noccaea* < 4.5%, Mummenhoff et al. (1997); *Cardamine* < 4.5%, Franzke et al. (1998)). Within the *Yinshania* clade, sequence distance values range up to 6%, but within the *Hilliella/Cochleariella* clade they range even higher, up to 27%.

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MOLECULAR CONFIRMATION OF UNIDIRECTIONAL HYBRIDIZATION IN *BEGONIA* × *TAIPEIENSIS* PENG (BEGONIACEAE) FROM TAIWAN[1]

Ching-I Peng[2] and Tzen-Yuh Chiang[3]

ABSTRACT

An unusual *Begonia* that sheds staminate flowers prematurely at bud stage was collected from several localities in northern Taiwan. Observations on morphology, pollen stainability, and seed set of this species initially suggested a hybrid origin. Morphological comparisons, distribution patterns, chromosome cytology, and experimental hybridization showed that such plants are F_1 hybrids ($2n = 41$) between *Begonia formosana* (Hayata) Masamune ($n = 30$) and *B. aptera* Blume ($n = 11$), both of which are widespread in Taiwan and sympatric in most of their ranges. These hybrids were named *Begonia* × *taipeiensis* Peng. Experimental crosses between the putative parental species consistently resulted in germinable seeds and healthy F_1 plants only when *B. formosana* was used as the female parent. Molecular data obtained from sequences of the *atp*B-*rbc*L spacer of chloroplast DNA confirmed that unidirectional hybridization between the putative parents in the wild resulted in the formation of *B.* × *taipeiensis*. No natural hybrid populations with a maternal origin from *B. aptera* have been detected. Abortion caused by a post-pollination barrier occurs when *B. aptera* was used as a maternal parent. Low pollen fertility of F_1 hybrids indicates that the natural hybrid is maintained by recurrent hybridization between the parental species.

Key words: *atp*B-*rbc*L noncoding spacer, *Begonia* × *taipeiensis*, *B. formosana*, *B. aptera*, chloroplast DNA, cytology, directional hybridization, natural hybrid, Taiwan.

Natural hybridization, one of the most influential processes that increases species diversity and stabilizes genetic complexity via genetic recombination or introgression (Arnold, 1992, 1993), occurs frequently in plants (P. S. Soltis & D. E. Soltis, 1991; P. S. Soltis et al., 1992; Arnold, 1997). Studies on the origins of natural hybrids and their genetic composition frequently raise fundamental questions concerning reproductive barriers, survivorship and fitness of the hybrids, and mechanisms of reticulate evolution. In many cases (e.g., Louisiana Irises; Arnold & Bennett, 1993) natural selection may favor hybrid plants of specific maternal origin. It is essential to study the genetic makeup of natural hybrids in order to elucidate evolutionary processes and the ecological adaptation of these plants.

It has been abundantly demonstrated that natural hybridization plays a major role in the evolution of species groups or complexes (*Ludwigia*: Peng, 1988, 1990; *Iris*: Arnold et al., 1990; *Glycine*: Doyle et al., 1990; *Helianthus*: Rieseberg et al., 1990, Rieseberg, 1991; *Gossypium*: Wendel et al., 1991; *Senecio*: Harris & Ingram, 1992; *Allium*: Ohsumi et al., 1993; *Leucaena*: Hughes & Harris, 1994; *Arabidopsis*: Mummenhoff & Hurka, 1995; *Argyroxiphium*: Baldwin, 1997; and *Argyranthemum*: Francisco-Ortega et al., 1997). Natural hybridization occurs frequently in Taiwanese *Begonia* (Y. K. Chen, 1988; Peng & Chen, 1991; Peng & Sue, 2000), which may have resulted in the high level of endemism of this genus on the island (66.7%; cf. C. H. Chen, 1993), while causing difficulties in clarifying phylogenetic relationships (see Rieseberg & Morefield, 1995).

Begonia × *taipeiensis* Peng was recently described as a new intersectional hybrid (Peng & Sue, 2000). Geographical distribution, low level of pollen stainability, and intermediate morphological characters between *B. formosana* (Hayata) Masamune and *B. aptera* Blume initially suggested such an origin. *Begonia aptera* [sect. *Sphenanthera* (Hassk.) Warb.] is distributed in northern Taiwan,

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while *B. formosana* [sect. *Platycentrum* (Klotzsch) A. DC.] is widespread in northern and eastern Taiwan. Both species occur in wide altitudinal ranges (50–2000 m). They frequently co-occur throughout their range of overlap (Fig. 1). The flowering periods are March to December for *B. formosana*, June to August for *B. aptera*, and April to December for *B.* × *taipeiensis*. Two discrete populations of *Begonia* × *taipeiensis* were found mixed with its putative parents in the Taipei Basin of northern Taiwan. *Begonia formosana* is characterized by having creeping rhizomes with ascending to erect flowering stems, broadly ovate, lobed leaves, 2-locular ovaries, and unequally winged capsules, the abaxial one being very pronounced. In contrast, *B. aptera* is a tall, cane species lacking horizontal rhizomes, has lanceolate leaves, 3-locular ovaries, and rounded, wingless capsules. *Begonia* × *taipeiensis* has short rhizomes, intermediate plant height and leaf shape, and 2-locular ovaries (these occasionally highly reduced and without locules) with diminutive wings. Like all other species of *Begonia*, *B.* × *taipeiensis* is monoecious and interflorally protandrous. At anthesis, it produces abundant flowers of both sexes. All staminate flowers observed develop normally up to their late bud stages and then drop off before they open. All *Begonia* of Taiwan are frequently pollinated by blowflies (Calliphoridae), hover flies (Syrphidae), and honeybees (Apidae) (Y. K. Chen, 1988). Plants of *Begonia* often grow along moist stream banks. Their light seeds float and are carried easily by water currents. Seeds of *Begonia* are primarily dispersed with the aid of wind (van der Pijl, 1972).

Traditionally, morphological examination and chromosome cytology have provided useful information in revealing putative parents. Nevertheless, such data are not sufficient to determine the direction of hybridization and to detect genetic variability. Recently, many convincing studies using molecular techniques such as comparison of RFLP patterns of ribosomal DNA (Arnold et al., 1990; Stein & Barrington, 1990; Rieseberg, 1991; P. S. Soltis & D. E. Soltis, 1991; Hughes & Harris, 1994; Mummenhoff & Hurka, 1995) and those of organelle DNA (D. E. Soltis & P. S. Soltis, 1989; DeMarais et al., 1992; Garcia & Davis, 1994; Wang & Szmidt, 1994; Brochmann et al., 1998), coupled with experimental hybridizations (Hodges et al., 1996), have been conducted to address reticulate or polyploid evolution (D. E. Soltis & P. S. Soltis, 1993). Chloroplast and mitochondrial genomes, being maternally inherited in most organisms (Chiu & Sears, 1985; D. E. Soltis et al., 1990), have been useful in elucidating the direction of gene flow between populations (Whittemore & Schaal, 1991) and in detecting the maternal origin of natural hybrids (Wendel et al., 1991). Thus, in this study, a noncoding spacer region between the genes *rbc*L and *atp*B of the chloroplast genome, which has been used as a molecular marker at the species level (Golenberg et al., 1993; Chiang, 1994; Manen & Natali, 1995; Chiang et al., 1998), was sequenced.

In this study the following questions will be addressed: (1) Did *B.* × *taipeiensis* originate from interspecific hybridization between *B. aptera* and *B. formosana*? (2) Do hybrids with alternate maternal origins have the same survivorship? (3) Do hybrids backcross with putative parents and produce viable offsprings? (4) Is the frequency of occurrence of natural hybrids a reflection of the ease of experimental crosses?

Materials and Methods

1. Materials sampled

Major herbaria in Taiwan (HAST, NTUF, TAI, TAIF, TNM) were consulted to determine the distribution and phenology of *Begonia* × *taipeiensis* and its putative parents, *B. formosana* and *B. aptera*. Living plants of the hybrid and its associated putative parents were collected from two localities of Taipei County, Hsichih and Wulai (Fig. 1). Additionally, a plant each of *B. formosana* and *B. aptera* from Chiufen and Nankang, respectively, where *B.* × *taipeiensis* was not found, were collected for comparison (Fig. 1). All plants were grown in the experimental greenhouse of the Institute of Botany, Academia Sinica, Taipei, for cytological and molecular examination and for experimental hybridization. *Begonia palmata* D. Don, a congener collected from Hsitou, Nantou County, was used as an outgroup. Herbarium vouchers were deposited at HAST (Table 1).

2. Experimental pollination

Experimental self-pollinations of *B. aptera* and *B. formosana* were done. Reciprocal crosses were carried out between *B. formosana* and *B. aptera* from the same locality and from different localities in the greenhouse. *Begonia* × *taipeiensis* collected from the wild was used as a pistillate parent for backcrossing with both putative parents. The fact that *Begonia* × *taipeiensis* shed staminate flowers prematurely precludes the possibility of using it as a pollen donor. Seeds (F_1 progeny) were grown to maturity to examine the fertility of pollen grains and for cytological study.

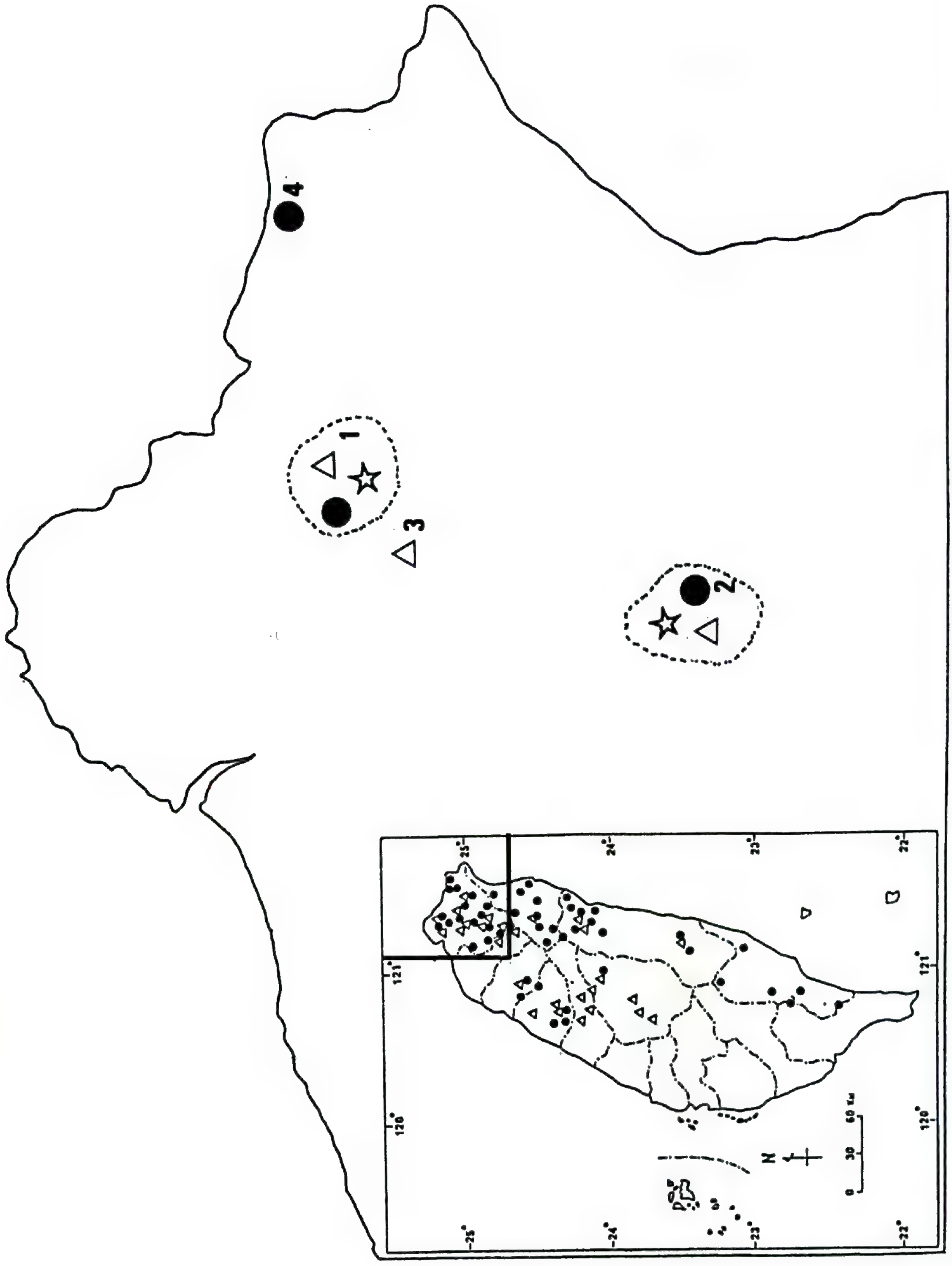

Figure 1. Map showing the distribution (inset) of *Begonia formosana* (circles) and *B. aptera* (triangles) in Taiwan; and location of the specimens collected from localities (1–4) for cytological and molecular studies: *B. formosana* (circles), *B. aptera* (triangles), and *B.* × *taipeiensis* (stars); 1, Wulai; 2, Hsichih; 3, Nankang; 4, Chiufen.

Table 1. Materials of *Begonia* × *taipeiensis* and its putative parents for cytological and molecular analysis. Herbarium vouchers are deposited at HAST.

Taxa	Localities	Vouchers	EMBL accession no.
B. × *taipeiensis*	1. Wulai, Taipei Co.	*Peng 13899*	AJ009601
		Peng 15106	AJ009602
	2. Hsichih, Taipei Co.	*Peng 16320*	AJ009600
B. aptera	1. Wulai, Taipei Co.	*Peng 16276*	AJ223092
		Peng 16153	AJ009606
	2. Hsichih, Taipei Co.	*Peng 16321*	AJ009605
	3. Nankang, Taipei Co.	*Peng 16292*	AJ009604
B. formosana	1. Wulai, Taipei Co.	*Peng 13915*	AJ009599
	2. Hsichih, Taipei Co.	*Peng 16319*	AJ009598
	3. Chiufen, Taipei Co.	*Leu 867*	AJ009597
B. palmata	1. Chitou, Nantou Co.	*Peng 16081*	AJ007745
	2. Tengchi, Kaohsiung Co.	*Peng 16831*	AJ242856
Experimental hybrid:			
Bf×*a*-1: *B. formosana* × *B. aptera* = *Leu 867* × *Peng 16153*			AJ242857
Bf×*a*-2: *B. formosana* × *B. aptera* = *Peng 13915* × *Peng 16153*			AJ242858

3. CHROMOSOME CYTOLOGY

Flower buds to be examined for meiotic behavior were fixed in a 3 : 1 mixture of 95% ethanol and glacial acetic acid and stored in the refrigerator. Prior to staining, the buds were hydrolyzed for 5–8 min. at 60°C using a 1 : 1 mixture of concentrated HCl and 95% ethanol. They were then squashed in FLP orcein (Jackson, 1973). Somatic chromosome counts were obtained from actively growing root tips pretreated for 3 to 4 hr. in 8-hydroxyquinoline, then fixed as above for at least 10 min. The root tips were then hydrolyzed in 1 N HCl for 8–10 min. at 60°C and squashed in FLP orcein. All analyzable chromosome configurations (mostly diakinesis or metaphase I) were documented with camera lucida drawings or photomicrographs using Kodak Panatomic-X films. Negatives and drawings are deposited at the Institute of Botany, Academia Sinica, Taipei.

4. MOLECULAR METHODS

DNA isolation, PCR, and nucleotide sequencing. Fresh tissue of young shoots was ground in liquid nitrogen and stored at −70°C for DNA extraction. Genomic DNA was extracted following a CTAB methodology (Doyle & Doyle, 1989). PCR was performed in a volume of 100 μl reaction using 10 ng of template DNA, 10 μl of 10× buffer, 10 μl $MgCl_2$ (25 mM), 10 μl dNTP mix (8 mM), 10 pmole of each primer, 10 μl of 10% NP-40, and 2 U of *Taq* polymerase (Promega, Madison, U.S.A.; cf. Chien et al., 1976). The reaction was programmed on an MJ Thermal Cycler (PTC 100) as one cycle of denaturation at 95°C for 4 min., 30 cycles of denaturation at 92°C for 45 sec., annealing at 52°C for 1 min. 15 sec., and extension at 72°C for 1 min. 30 sec., followed by a 10 min. extension. Template DNA was denatured with reaction buffer, $MgCl_2$, NP-40, and ddH2O for 4 min. (first cycle), and cooled on ice immediately. Primers [*rbc*L-1, 5′-AACACCAGCTTTA(G)AATCCAA-3′; *atp*B-1, ACATCT(G)AA(G)TACT(G)GGACCAATAA-3′; (Chiang et al., 1998)], dNTPs, and *Taq* polymerase were added to the above ice-cold mix. Reaction was restarted at the first annealing at 52°C. PCR fragments were eluted using High Pure PCR Product Purification Kit (Boehringer Mannheim, Germany; cf. Vogelstein & Gillespie, 1979). PCR products were ligated to a pT7blue T-vector (Novagen, Madison, U.S.A.; cf. Marchuk et al., 1991) and cloned in competent *E. coli* DH5α. Plasmid DNA was extracted from transformed cells and sequenced with ^{32}P labeling extension/termination reaction (Sanger et al., 1977) using *fmol* DNA Sequencing System (Promega, Madison, U.S.A.; cf. Murray, 1989). For completing sequencing, with about 75 base pair overlaps from both ends, two additional primers, i.e., *rbc*L-*2* (5′-GGTTCGTTCTTTGAACATGG-3′) and *atp*B-*2* (5′-CCGTCCAACCGAATCCAATTC-3′), were synthesized.

DNA alignment and phylogenetic analysis. Alignment of nucleotide sequences was performed by Clustal V program (Higgins et al., 1992) and improved by eye. Parsimony phylogenetic anal-

yses were performed by using the Phylogenetic Analysis Using Parsimony Program (PAUP, version 3.1.1., Swofford, 1993) with the branch and bound algorithm. Neighbor-Joining (NJ) analysis by calculating Kimura's (1980) two-parameter distance was also performed using Molecular Evolutionary Genetics Analysis Program (MEGA, version 1.01, Kumar et al., 1993). Confidence of clades reconstructed was tested by bootstrapping (Felsenstein, 1985) with 1000 replicates (Hedges, 1992). The nodes with bootstrap values greater than 0.70 are significantly supported with > 95% probability (Hillis & Bull, 1993). In NJ analysis, complete-and-partial (CP) bootstrap technique was used to correct the bias (conservativeness) of the standard bootstrap approach (Li & Zharkikh, 1995). A g1 test (Huelsenbeck, 1991) of skewed tree-length distribution was calculated from 10,000 random trees generated by PAUP in order to measure the information content of the data. Critical values of the g1 test were obtained from Hillis and Huelsenbeck (1992). The fit of character data on phylogenetic hypotheses (Swofford, 1991) was evaluated by the consistency index, CI (Kluge & Farris, 1969), and the retention index, RI (Farris, 1989). The statistical significance of the CI was determined according to the method of Klassen et al. (1991). The number of nucleotide substitution, which is the number of transitional and transversional substitutions per site, was calculated following the methodology of Wu and Li (1985).

Results

EXPERIMENTAL SELFING AND HYBRIDIZATION

In all experimental geitonogamous selfing attempts made on *Begonia formosana* and *B. aptera*, fruits with 95–100% viable seeds were consistently obtained. Plants of *B. formosana* required 30–45 days for fruit maturation, whereas those of *B. aptera* required 90–150 days. *Begonia formosana* and *B. aptera* were crossed reciprocally in the experimental greenhouse. Nearly all crosses using *B. formosana* as pistillate parent were successful, producing mature fruits with 80–90% plump seeds 35–45 days after pollination. Such seeds were viable and flowering artificial F_1 hybrids were readily obtained from them. The level of stainable pollen in these F_1 plants was extremely low (ranging from 0 to 5%), which agrees with that of the naturally occurring *B.* × *taipeiensis*. When *B. aptera* was used as the pistillate parent in the crosses, however, precocious fruit drop occurred ca. 60 days after artificial pollination.

Experimental hybrids between *B. formosana* and *B. aptera* were grown to maturity. They flowered annually and have persisted in the mist greenhouse since the summer of 1995. The plant height in these artificial hybrids was within the variation range of naturally occurring *B.* × *taipeiensis*. The artificial hybrids closely resembled *B.* × *taipeiensis* in habit and details of vegetative as well as floral characters. When plants of *Begonia* × *taipeiensis* collected from the wild were backcrossed (as pistillate parent) to *B. formosana* and *B. aptera*, respectively, fruit set was successfully obtained. However, five backcrossing attempts made with *B. formosana* and 11 such attempts with *B. aptera* produced fruits with zero or negligible plump seeds that failed to germinate.

CHROMOSOME CYTOLOGY

Previous studies revealed a meiotic chromosome number of $n = 11$ in *B. aptera*, $n = 30$ in *B. formosana*, and $2n = 41$ in *B.* × *taipeiensis* (Peng & Sue, 2000). Like *B.* × *taipeiensis*, experimental hybrids of *B. formosana* × *B. aptera* consistently have a somatic chromosome number of $2n = 41$. Also, abnormalities in chromosome configurations were observed. Meiotic chromosome configurations of both *B.* × *taipeiensis* and *B. formosana* × *B. aptera* typically consisted of some sticky, often disoriented bivalents, univalents, and multivalent associations (Fig. 2).

MOLECULAR DATA

Sequences with a consensus length of 854 base pairs of the *atp*B-*rbc*L spacer were obtained from *B.* × *taipeiensis* and its putative parents. Fifteen variable sites were found between sequences (Table 2). That this chloroplast spacer has, on average, 34.0% A and 36.1% T agrees with one of the common properties, i.e., AT-rich, of most noncoding spacers (Li, 1997). Differences in the rate of nucleotide substitution (Table 3) among species of *Begonia* we sampled ranged from 0.0017 (between *B. aptera* and *B. formosana*) to 0.0056 (between *B. aptera* and *B. palmata*) (mean = 0.0033).

Of the three individuals of *B. formosana* we examined, two had identical nucleotide sequences (Tables 1, 2). The third collection from Chiufen (*Leu 867*) differed at two different positions (bp 191 and 762). Interpopulational variation was present in *B. aptera* at bp 275 and 794. In our study, sequences of this chloroplast spacer for all three species were highly conserved, with 15 variable sites (2.1% of 854 bp) (Table 2). *Begonia formosana* shared with *B. aptera* 7 derived characters (at sites 43, 44, 251, 252, 280, 568, 743). Two autapomor-

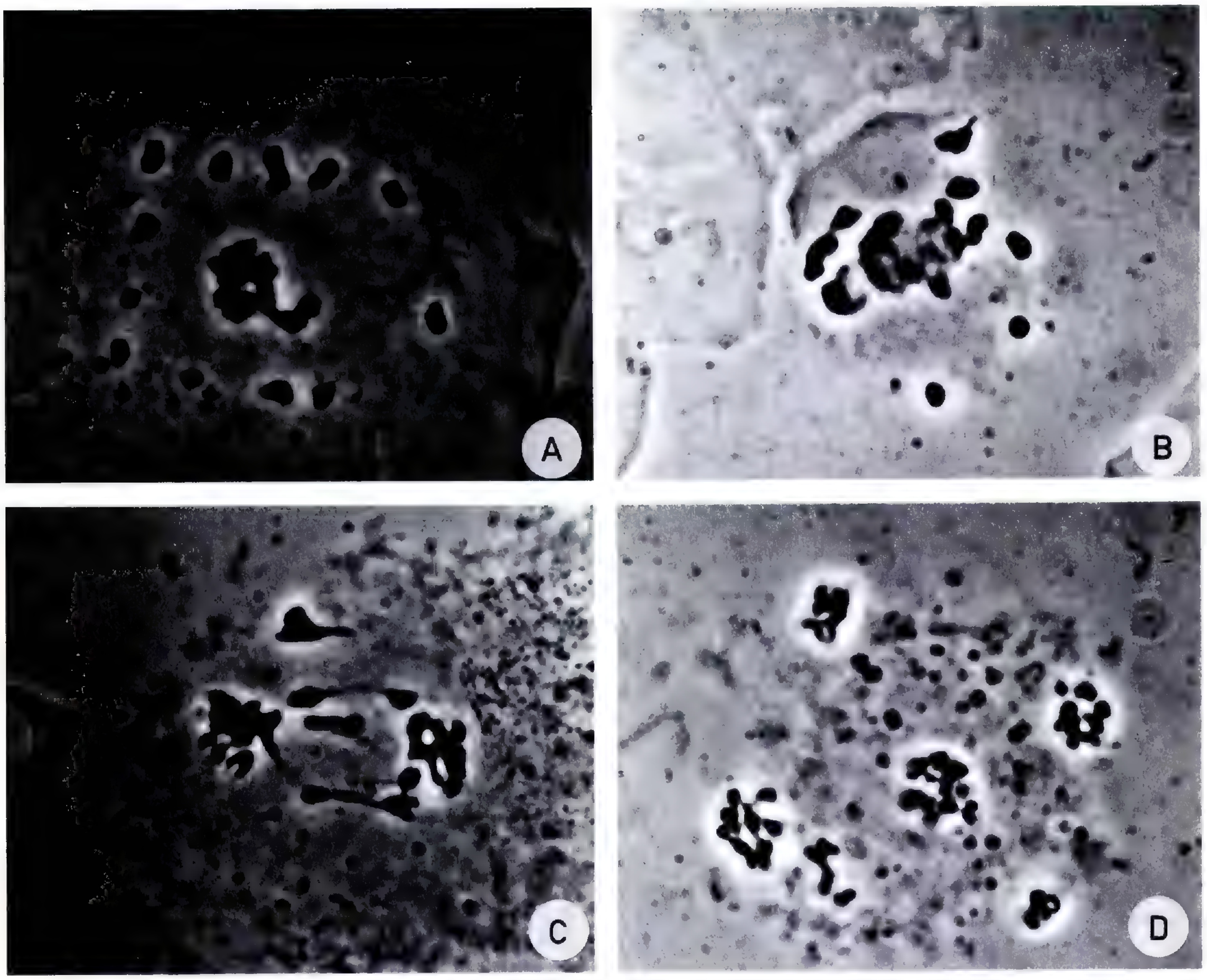

Figure 2. Meiotic chromosome spreads of *Begonia*.—A. Metaphase I, polar view. —B. Metaphase I, equatorial view. —C. Anaphase I. —D. Telophase II. Bar equals 10 μm (A, *Begonia* × *taipeiensis*, from *Peng 13899*; B–D, *B. formosana* × *B. aptera*, from *Leu 867* × *Peng 16153*).

Table 2. Variable sites of the nucleotide sequences of the *atp*B-*rbc*L spacer region of the chloroplast DNA of *Begonia* species. Dots indicate that the nucleotide sequences are identical to those for *Begonia aptera 16321*.

Taxa/Sites	43	44	191	251	252	270	275	286	298	371	376	568	743	762	794
B. aptera 16321	C	G	T	T	A	A	A	A	G	T	T	C	T	T	G
B. aptera 16276	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
B. aptera 16153	.	.	.	.	.	.	G	.	.	.	.	.	.	.	C
B. aptera 16292	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
B. taipeiensis 15106	.	.	.	.	.	.	.	.	A	G	C	.	.	.	.
B. taipeiensis 13899	.	.	.	.	.	.	.	.	A	G	C	.	.	.	.
B. taipeiensis 16320	.	.	.	.	.	.	.	.	A	G	C	.	.	.	.
Bf×*a*-2	.	.	.	.	.	.	.	.	A	G	C	.	.	.	.
Bf×*a*-1	.	.	C	.	.	.	.	.	A	G	C	.	.	C	.
B. formosana 13915	.	.	.	.	.	.	.	.	A	G	C	.	.	.	.
B. formosana 16319	.	.	.	.	.	.	.	.	A	G	C	.	.	.	.
B. formosana 867	.	.	C	.	.	.	.	.	A	G	C	.	.	C	.
B. palmata 16081	T	A	C	A	C	T	.	C	A	.	.	T	A	.	.
B. palmata 16831	T	A	C	.	C	T	.	C	A	.	.	T	A	.	.

Table 3. Pairwise comparisons of the numbers of nucleotide subsitutions per site (K) in *Begonia* species (above diagonal). 1. *B. aptera 16321*; 2. *B. aptera 16276*; 3. *B. aptera 16153*; 4. *B. aptera 16292*; 5. *B. taipeiensis 15106*; 6. *B. taipeiensis 13899*; 7. *B. taipeiensis 16320*; 8. *Bf*×*a-2*; 9. *Bf*×*a-1*; 10. *B. formosana 13915*; 11. *B. formosana 16319*; 12. *B. formosana 867*; 13. *B. palmata 16081*; 14. *B. palmata 16831*.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1		0.0000	0.0011	0.0000	0.0017	0.0017	0.0017	0.0017	0.0028	0.0017	0.0017	0.0028	0.0056	0.0050
2			0.0011	0.0000	0.0017	0.0017	0.0017	0.0017	0.0028	0.0017	0.0017	0.0028	0.0056	0.0050
3				0.0011	0.0028	0.0028	0.0028	0.0028	0.0039	0.0028	0.0028	0.0039	0.0067	0.0061
4					0.0017	0.0017	0.0017	0.0017	0.0028	0.0017	0.0017	0.0028	0.0056	0.0050
5						0.0000	0.0000	0.0000	0.0011	0.0000	0.0000	0.0011	0.0061	0.0056
6							0.0000	0.0000	0.0011	0.0000	0.0000	0.0011	0.0061	0.0056
7								0.0000	0.0011	0.0000	0.0000	0.0011	0.0061	0.0056
8									0.0011	0.0000	0.0000	0.0011	0.0061	0.0056
9										0.0011	0.0011	0.0000	0.0061	0.0056
10											0.0000	0.0011	0.0061	0.0056
11												0.0011	0.0061	0.0056
12													0.0061	0.0056
13														0.0006

phies, at positions 371 and 376, in *B. formosana* distinguished it from *B. aptera*.

The sequences of *Leu 867* and *Peng 13915*, both *B. formosana*, differed at two sites. The genetic uniqueness of the two collections was transmitted to the F_1 offspring, i.e., B*f*×*a*-1 and B*f*×*a*-2, respectively, when they were used as maternal parent in experimental crosses. We sampled two collections of *Begonia* × *taipeiensis*, *Peng 13899* and *15106*, both associated with *B. formosana*, *Peng 13915*, from the same locality. Both *Begonia* × *taipeiensis* specimens were found to have identical sequences (Table 2) with the experimental hybrid (B*f*×*a*-2).

Parsimony analysis identified two equally parsimonious trees of 16 steps (Fig. 3), a CI of 0.938 ($P < 0.01$), and an RI of 0.958. A g1 statistic of −1.468 indicated a significant signal ($P < 0.01$). The Neighbor-Joining tree (Fig. 4), recovered by MEGA, based on the Kimura's two-parameter distance (Table 4), is consistent with the parsimony trees, but with higher resolution. Three major clades in both parsimony and NJ trees (Figs. 3, 4) obtained in these analyses were supported significantly by bootstrapping: the clade of *B. formosana*, *B.* × *taipeiensis*, and an experimental hybrid (B*f*×*a*-2), the clade of *B. formosana* (*Leu 867*) and an experimental hybrid (B*f*×*a*-1), and the clade of *B. aptera*. Within the first clade the monophyly of the subgroup composing the maternal parent (*B. formosana, Peng 16319*), the hybird offspring (B*f*×*a*-1), and another *B. formosana* (*Peng 13915*) was also significantly supported.

Discussion

Hybrid origin in *B.* × *taipeiensis*: evidence from morphology, cytology, and distribution

Based on morphological criteria, *B. formosana* and *B. aptera* were initially suggested as the putative parents of *B.* × *taipeiensis* (Peng & Sue, 2000). Cytological data showed that both the experimental hybrids between *B. formosana* ($n = 30$) and *B. aptera* ($n = 11$) and the naturally occurring *B.* × *taipeiensis* have the same chromosome number of $2n = 41$. *Begonia formosana* is the only species with $n = 30$ in Taiwan, which is the highest chromosome number among these *Begonia*. Chromosome numbers of $n = 11$, 15, 18, and 19 are known for other members of *Begonia* on the island (Y. K. Chen, 1988). A chromosome number of $n = 11$ is documented in only two species, *B. aptera* and *B. palmata* (Y. K. Chen, 1988; Peng & Chen, 1991). *Begonia palmata* was excluded as a candidate for a putative parent of *B.* × *taipeiensis* based on the geographic distribution of the species. Extensive fieldwork and examination of herbarium material revealed that the ranges of *B. aptera* and *B. formosana* largely overlap and that plants of *B.* × *taipeiensis* usually co-occur with these two species at ca. 60–200 m in elevation. On the other hand, *B. palmata*, mainly a montane species of higher altitudes (ca. 600–2100 m), is geographically and altitudinally disjunct. Based on data from experimental crosses, cytological observation, and geographical distribution, we conclude that *B.* × *taipeiensis* represents F1 progeny from natural hybridization between *B. formosana* and *B. aptera*.

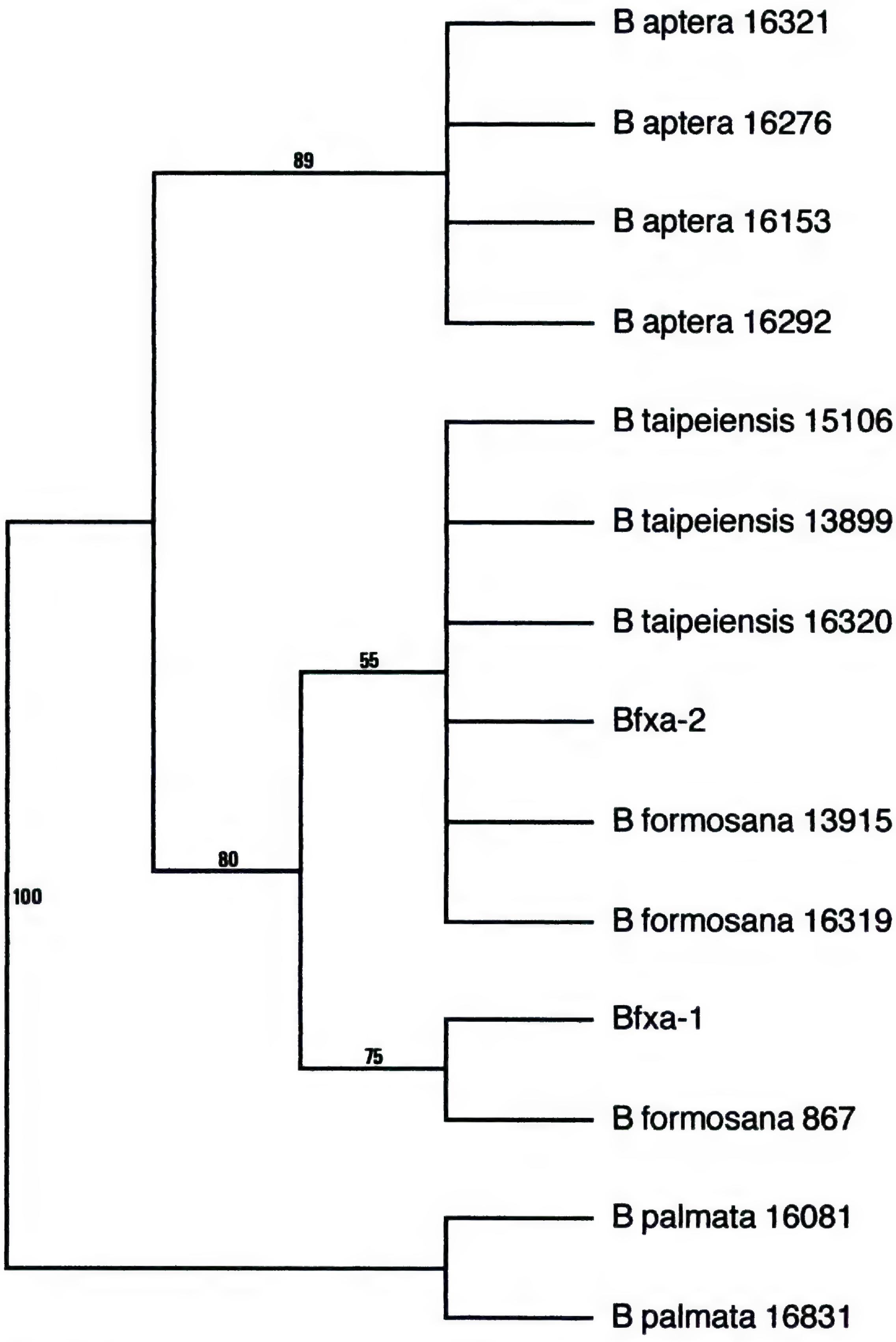

Figure 3. One of the parsimonious trees recovered by PAUP from nucleotide sequences of the *atp*B-*rbc*L spacer of chloroplast DNA rooted at *Begonia palmata*. Numbers at nodes are bootstrap values.

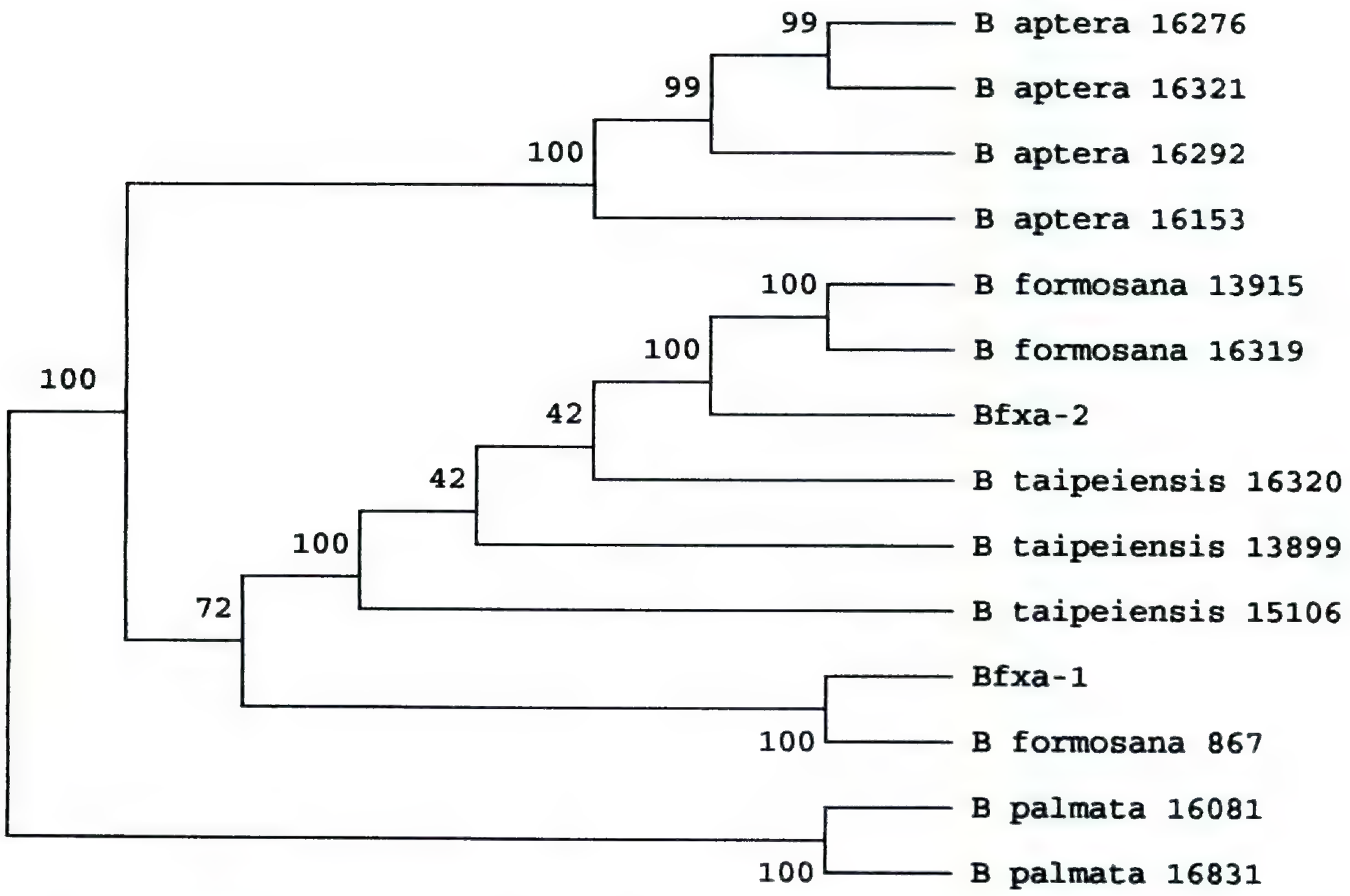

Figure 4. Neighbor-Joining tree recovered by MEGA from nucleotide sequences of *atp*B-*rbc*L spacer of chloroplast DNA rooted at *Begonia palmata*. Numbers at nodes are complete-and-partial (CP) bootstrap values.

UNIDIRECTIONAL HYBRIDIZATION GIVING RISE TO *B.* × *TAIPEIENSIS*: EVIDENCE FROM MOLECULAR DATA AND EXPERIMENTAL HYBRIDIZATION

Identical sequences of the chloroplast *atp*B-*rbc*L spacer between *B.* × *taipeiensis* and *B. formosana* suggested that the former had a maternal origin from the latter. Unidirectional hybridization giving rise to the natural hybrid *B.* × *taipeiensis* is congruent with the results from reciprocal crosses in which viable F_1's were obtained only when *B. formosana* was used as the pistillate parent. Experimental crosses using *B. aptera* as the maternal parent resulted in precocious fruit drop, possibly as a result of genetic disharmonies. A similar observation was made on Lousiana irises (Arnold & Bennett, 1993), in which unidirectional hybridization was documented using cpDNA haplotypes. Naturally occurring unidirectional hybridization suggests that hybrids of reversed parentages may have different survivorship, because organelle genomes may contribute genetic information that critically affects the survivorship of their progeny.

Compared to other organisms, such as in Hylocomiaceae (K = 0.012; Chiang, 1994) and Rubiaceae (K = 0.027; Manen & Natali, 1995), the chloroplast genetic variation between species of *Begonia* appears to be low. The distinctness of *B. formosana* supported by two derived sites (371 and 376) and that of *B. aptera* supported by a shared derived site (298) indicated, however, that this spacer is an appropriate marker at species level. The low level of interspecific variation in chloroplast DNA in Taiwanese *Begonia* may be ascribed to the abundant and recent natural hybridization and introgression events (Liu, 1999), which facilitated the morphological evolution and evolution of nuclear DNA via genetic recombination (based on our preliminary assessment of RAPD and nrDNA ITS sequence data). The possibility that the evolutionary rate of morphological changes may have surpassed that of the chloroplast DNA was similarly demonstrated in oaks with frequent natural hybridization (Whittemore & Schaal, 1991).

FIT OR UNFIT, AND RECURRENT HYBRIDIZATION OF *B.* × *TAIPEIENSIS*

Natural hybrids may be considered to be impoverished genetically (cf. D. E. Soltis & P. S. Soltis, 1993; Arft & Ranker, 1998). Whether natural hybrids are more or less fit relative to their parents is a controversial issue (Arnold & Hodges, 1995). Experimental hybridization in this study revealed that there are very few if any pre- and post-zygotic reproductive barriers between these *Begonia*. We

Table 4. Kimura 2-parameter distance (above diagonal) and standard errors (below diagonal) of nucleotide sequences of *atp*B-*rbc*L noncoding spacer in *Begonia*. 1. *B. aptera 16321*; 2. *B. aptera 16276*; 3. *B. aptera 16153*; 4. *B. aptera 16292*; 5. *B. taipeiensis 15106*; 6. *B. taipeiensis 13899*; 7. *B. taipeiensis 16320*; 8. *Bf*×*a-2*; 9. *Bf*×*a-1*; 10. *B. formosana 13915*; 11. *B. formosana 16319*; 12. *B. formosana 867*; 13. *B. palmata 16081*; 14. *B. palmata 16831*.

OTUs	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1		0.0000	0.0024	0.0000	0.0035	0.0035	0.0035	0.0035	0.0059	0.0035	0.0035	0.0059	0.0118	0.0106
2	0.0000		0.0024	0.0000	0.0035	0.0035	0.0035	0.0035	0.0059	0.0035	0.0035	0.0059	0.0118	0.0106
3	0.0017	0.0017		0.0024	0.0059	0.0059	0.0059	0.0059	0.0083	0.0059	0.0059	0.0083	0.0142	0.0130
4	0.0000	0.0000	0.0017		0.0035	0.0035	0.0035	0.0035	0.0059	0.0035	0.0035	0.0059	0.0118	0.0106
5	0.0020	0.0020	0.0026	0.0020		0.0000	0.0000	0.0000	0.0024	0.0000	0.0000	0.0024	0.0130	0.0118
6	0.0020	0.0020	0.0026	0.0020	0.0000		0.0000	0.0000	0.0024	0.0000	0.0000	0.0024	0.0130	0.0118
7	0.0020	0.0020	0.0026	0.0020	0.0000	0.0000		0.0000	0.0024	0.0000	0.0000	0.0024	0.0130	0.0118
8	0.0020	0.0020	0.0026	0.0020	0.0000	0.0000	0.0000		0.0024	0.0000	0.0000	0.0024	0.0130	0.0118
9	0.0026	0.0026	0.0031	0.0026	0.0017	0.0017	0.0017	0.0017		0.0024	0.0024	0.0000	0.0130	0.0118
10	0.0020	0.0020	0.0026	0.0020	0.0000	0.0000	0.0000	0.0000	0.0017		0.0000	0.0024	0.0130	0.0118
11	0.0020	0.0020	0.0026	0.0020	0.0000	0.0000	0.0000	0.0000	0.0017	0.0000		0.0024	0.0130	0.0118
12	0.0026	0.0026	0.0031	0.0026	0.0017	0.0017	0.0017	0.0017	0.0000	0.0017	0.0017		0.0130	0.0118
13	0.0037	0.0037	0.0041	0.0037	0.0039	0.0039	0.0039	0.0039	0.0039	0.0039	0.0039	0.0039		0.0012
14	0.0036	0.0036	0.0039	0.0036	0.0037	0.0037	0.0037	0.0037	0.0037	0.0037	0.0037	0.0037	0.0012	

would expect natural hybrids when species of *Begonia*, such as *B. aptera* and *B. formosana*, co-occur and have overlapping flowering periods. *Begonia* taxa are also well known for their capability for vegetative propagation: they easily proliferate from fragments of stems, rhizomes, or leaves. Hybrid populations of *B.* × *taipeiensis*, however, are of sporadic occurrence (Fig. 1). Furthermore, such hybrids drop staminate flowers precociously and are completely seed-sterile. Their sterility, small population size, and rare occurrence suggest that they are less fit than the parental species, *B. aptera* and *B. formosana*. Many recent studies also indicate that natural hybrids are usually less competitive than their parental species, unless a novel niche can be explored (Arnold, 1993; Arnold & Hodges, 1995).

Begonia × *taipeiensis* was not known until very recently (Peng & Sue, 2000). It is seed-sterile, of limited distribution, and co-occurs with its parental species. Because it sheds staminate flowers precociously, we used it as an ovule donor to make experimental backcrosses with both of its parental species to test the possiblilty of introgression. Such attempts consistently failed to produce viable seeds. This, plus the sterility in *B.* × *taipeiensis*, led us to suggest that it can only persist through recurrent hybridization. Many recent molecular studies also suggest that recurrent hybridization events may occur over short spans of time (Ashton & Abbott, 1992; D. E. Soltis & P. S. Soltis, 1993; Arft & Ranker, 1998).

Although *B.* × *taipeiensis* is highly sterile, significant genetic variability could have been incorporated and accumulated into this hybrid from genetically distinct parental species (cf. Arft & Ranker, 1998). In nature, *B.* × *taipeiensis* with low frequency of fertility, which may not have been detected due to limited sampling, may occur. Similarly, the low level of viable seeds in backcrossings did not rule out the possibility of introgression in wild populations. Further study with wider and more intense samplings will be able to provide insight into the impact or potential of rarely occurring events on the evolution of Taiwanese *Begonia*.

In conclusion, when analyzed in concert, the data suggest that the formation of the natural hybrid *B.* × *taipeiensis* occurs via pollen transfer from *B. aptera* to the maternal species, *B. formosana*. Unidirectional hybridization suggests that differential survivorship exists between hybrids with reversed maternal origins. Even considering the ease with which experimental crosses are obtained, natural hybrids appear less fit than the parental species in this study, based on their sterility.

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VEGETATION OF LIMESTONE AND DOLOMITE GLADES IN THE OZARKS AND MIDWEST REGIONS OF THE UNITED STATES[1]

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ABSTRACT

Literature on the vegetation of limestone and dolomite (cedar) glades in the Ozarks of Missouri and Arkansas and in the midwestern United States (Illinois, Indiana, Ohio, Wisconsin) is reviewed. Dominant plants in these glades are C_4 perennial prairie grasses, of which little bluestem (*Schizachyrium scoparium* (Michx.) Nash) is the most important. Without removal of invading woody plants by fire or other means, succession in these rocky, calcareous openings is to forest. They differ from cedar glades in the southeastern United States, which are dominated by C_4 annual grasses (primarily *Sporobolus vaginiflorus* (Torr. ex Gray) Wood) and do not require management or natural disturbances to maintain them. We suggest that the anthropogenic, prairie-grass-dominated openings in the Ozarks and Midwest be called xeric limestone (or dolomite) prairies and that the term cedar glades be used for an edaphic climax dominated by C_4 summer annual grasses in natural openings on limestone or dolomite bedrock.

Key words: annual *Sporobolus* species, Midwestern (U.S.A.) limestone glades, Ozark glades, *Schizachyrium scoparium*, woody plant invasion, xeric limestone prairies

Although the climatic climax vegetation in the eastern United States is forest (Shreve, 1917; Braun, 1950; Küchler, 1964), long-persisting plant communities dominated by herbaceous angiosperms and/or cryptogams occur in areas where bedrock is exposed and/or soil depth in most places is too shallow to support trees or shrubs. Examples of these edaphic climax rock outcrop communities include the mid-Appalachian shale barrens (Platt, 1951; Keener, 1983; Braunschweig et al., 1999), granite outcrops of the southeastern piedmont (Oosting & Anderson, 1939; McVaugh, 1943; Keever et al., 1951; Burbanck & Platt, 1964; Palmer, 1970; Shure, 1999), and the cedar (limestone) glades of the Central (Nashville) Basin in Tennessee (Quarterman, 1950; Baskin & Baskin, 1985; Somers et al., 1986; Drew, 1991) and of the southeastern United States in general (Baskin & Baskin, 1999).

The terms "cedar glades" and "limestone (or dolomite) glades" are also used to describe grass/forb-dominated openings over shallow limestone or dolomite soil on ridgetops and side slopes in the Ozarks (Steyermark, 1940; Erickson et al., 1942; Kucera & Martin, 1957; Ladd & Nelson, 1982; Nelson & Ladd, 1983; Nelson, 1985; Fig. 1) and Midwest (e.g., Curtis, 1959; Kurz, 1981; Aldrich et al., 1982; Heikens & Robertson, 1995). Thus, the use of these terms in the Ozarks (Arkansas, Missouri), Midwest (Illinois, Indiana, Ohio, Wisconsin), and Southeast (see fig. 12.2 (map), p. 208 in Baskin & Baskin, 1999) has been interpreted to mean that cedar glade vegetation is the same in the three regions (cf. Curtis, 1959; Küchler, 1964). However, cedar glades of the southeastern United States are dominated by C_4 summer annual grasses, primarily *Sporobolus vaginiflorus*, and those of the Ozarks/Midwest are dominated by C_4 perennial grasses, primarily *Schizachyrium scoparium* (Baskin et al., 1994, 1995). In a recent review of the literature on cedar glades of the southeastern United States, Baskin and Baskin (1999) concluded that: (1) *Sporobolus vaginiflorus* is the most important species in this limestone/dolomite rock outcrop vegetation type; and (2) neither *Schizachyrium scoparium* nor any other perennial grass species is an important component of the vegetation. No such review of quantitative information has been published on the vegetation of calcareous glades in the Ozarks and/or Midwest.

Thus, the purpose of this paper is to review the literature on the vegetation of limestone and dolomite glades in the Ozarks and Midwest. In particular, quantitative and/or qualitative evidence is presented that, indeed, *Schizachyrium scoparium* is

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Figure 1. *Top,* Valley View Glade, Jefferson County, Missouri. *Bottom,* a glade in the Hercules Glades Wilderness Area, Taney County, Missouri. Photographs taken by Carol C. Baskin on 24 July 1996 (top) and 26 July 1993 (bottom).

the dominant plant species of these calcareous glades and that annual grasses are relatively unimportant. In addition, this review contrasts the calcareous glades of the Ozarks and Midwest with those of the southeastern United States.

The Ozarks

Steyermark (1940; see also Steyermark, 1959) recognized five climax vegetation associations (sensu the polyclimax theory) of the Missouri Ozarks and qualitatively described stages in primary succession leading to each of them. He also recognized several subclimaxes in these seres. However, these presumed successional sequences probably represent vegetation sequences along environmental gradients that are unrelated to succession per se.

The open limestone and dolomite glades of the Missouri Ozarks are the first two stages of what Steyermark (1940) presented as a six-stage sere beginning on bare, rocky slopes and ending with a sugar maple–white oak climatic climax: (1) *Bouteloua curtipendula* (Michx.) Torr.–*Rudbeckia missouriensis* Engelm.; (2) *Rhus aromatica* Ait.–*Diospyros virginiana* L.–*Juniperus virginiana* L. (with a redcedar subclimax persisting on eroded limestone slopes and knobs); (3) *Bumelia lanuginosa* (Michx.) Pers.–*Viburnum rufidulum* Raf.; (4) *Ulmus alata* Michx.–*Rhamnus caroliniana* Walt.; (5) *Quercus muhlenbergii* Engelm.–*Fraxinus americana* L. (with *Q. muhlenbergii* forming a subclimax on southern and western exposures); and (6) *Acer saccharum* Marsh.–*Quercus alba* L. Climax vegetation in the White River region of southwestern Missouri may be *Q. muhlenbergii–Cotinus obovatus* Raf. Thus, the pioneer stage in this sere is a limestone (or dolomite) glade (sensu Steyermark, 1940: 392) ". . . with largely a component of prairie species. . . ." The dominants are *Bouteloua curtipendula* and *Rudbeckia missouriensis* (*B. curtipendula, Psoralidium tenuiflorum* (Pursh) Rydb., and *Silphium laciniatum* L., or *Andropogon gerardii* Vit. and *B. curtipendula* in some parts of the Ozarks). In addition to *A. gerardii* and *B. curtipendula,* other important C_4 grasses on these glades are: *Panicum virgatum* L., *Sorghastrum nutans* (L.) Nash, *Schizachyrium scoparium,* and *Sporobolus neglectus* Nash. Important forbs include *Agave virginica* (L.) Rose, *Allium stellatum* Roth., *Aster oblongifolius* Nutt., *Calamintha arkansana* (Nutt.) Shinn., *Dalea purpurea* Vent., *Echinacea pallida* (Nutt.) Nutt., *E. paradoxa* (Norton) Britt. var. *paradoxa* (an Ozark cedar glade endemic), *Hedyotis nigricans* (Lam.) Fosb., *Heliotropium tenellum* (Nutt.) Torr., *Oenothera macrocarpa* Nutt. subsp. *macrocarpa,* and *Polytaenia nuttallii* DC. *Berchemia scandens* (Hill) K. Koch is a common woody vine in this pioneer stage in the southwestern Ozarks.

A second seral stage begins when *Rhus aromatica, Diospyros virginiana, Juniperus virginiana,* and other woody plants invade these limestone (or dolomite) glades. *Juniperus virginiana* typically becomes the most conspicuous plant, especially on eroded slopes and "bald knobs," forming what Steyermark referred to as "cedar glades," "red cedar glades," or "red cedar balds." These terms indicate scattered redcedar trees in a matrix of herbaceous vegetation dominated by prairie grasses, in particular *A. gerardii, P. virgatum,* and *S. scoparium.* In the White River region of southwestern Missouri, *J. virginiana* and/or *D. virginiana* may invade limestone glades and become the dominant species. *Sassafras albidum* (Nutt.) Nees may co-occur with *D. virginiana* as a pioneer woody species.

Other investigators also have found that prairie grasses are the dominants of cedar glades in the Ozarks of Missouri. Kucera and Martin (1957) reported *Schizachyrium scoparium* as the dominant species (85% frequency, 51% of herbaceous cover) in cedar glades in the Ozarks of southwestern Missouri. Locally dominant grasses included *A. gerardii, B. curtipendula, P. virgatum, Sorghastrum nutans, Sporobolus heterolepis* (A. Gray) A. Gray, *Tridens flavus* (L.) A. Hitchc., and the C_4 summer annual *Sporobolus neglectus. Hedyotis nigricans* was the most important herbaceous dicot, *Rhus aromatica* the most common shrub, and *Juniperus virginiana* the most common tree. Other typical woody species included *Bumelia lanuginosa, Cotinus obovatus, Diospyros virginiana, Ilex decidua* Walt., and *Rhamnus caroliniana* Walt. Hicks's (1981) results of a vegetational analysis of seven stands of open cedar glades with < 30% woody plant canopy cover (stages 1 and 2 in Steyermark's scheme of xerarch succession to a sugar maple–white oak climax) in the Hercules Glades Wilderness Area in southwestern Missouri were quite similar to those of Kucera and Martin (1957). *Schizachyrium scoparium* was the dominant species, and *H. nigricans, Rhus aromatica,* and *J. virginiana* were the most important herbaceous dicot, shrub, and tree, respectively (Table 1). *Schizachyrium scoparium* had the highest percent importance value (%I.V.) in all seven stands sampled by Hicks.

Hall (1955) determined frequency of herbs + seedlings and of sprouts of woody plants 0.27 m or less in height and the number of *Juniperus virginiana* seedlings/saplings in an open dolomite glade in the Missouri Botanical Garden's Shaw Arboretum, near the northeastern border of the Ozark Pla-

Table 1. Average percent importance values (%I.V.) for herbs, shrubs/vines, and trees in seven stands of open cedar glades in the Ozarks of southwestern Missouri. Only plants with a %I.V. of ≥ 1.0% are listed. Percent constancy and %I.V. were calculated from data in J. L. Hicks (1981).

Species	% constancy	%I.V
HERBS		
Schizachyrium scoparium (Michx.) Nash	100	29.21
Hedyotis nigricans (Lam.) Fosb.	100	10.47
Sporobolus neglectus Nash	100	9.47
Carex sp.	100	7.37
Rudbeckia missouriensis Engelm.	100	7.30
Panicum virgatum L.	100	6.40
Croton capitatus Michx./*C. monanthogynus* Michx.	100	5.10
Sorghastrum nutans (L.) Nash	100	3.81
Andropogon gerardii Vit.	71	2.57
Tragia betonicifolia Nutt./*T. ramosa* Torr.	100	1.73
Silphium terebinthinaceum Jacq.	71	1.51
Sporobolus heterolepis (A. Gray) A. Gray	86	1.44
Palafoxia callosa (Nutt.) Torr. & A. Gray	71	1.41
Evolvulus nuttallianus Roemer & Schultes	57	1.31
Heliotropium tenellum (Nutt.) Torr.	86	1.31
Oenothera macrocarpa Nutt. subsp. *macrocarpa*	86	1.17
Dalea purpurea Vent.	86	1.11
104 other species		9.18
	Total	101.87
SHRUBS/VINES		
Rhus aromatica Ait.	100	58.77
Symphoricarpos orbiculatus Moench	86	9.71
Berchemia scandens (Hill) K. Koch	43	7.24
Rosa setigera Michx.	57	5.40
Andrachne phyllanthoides (Nutt.) J. Coulter	14	5.34
Mimosa quadrivalvis L.	29	4.99
Vitis aestivalis Michx.	57	4.84
Smilax bona-nox L.	57	2.10
Parthenocissus quinquefolia (L.) Planch.	29	1.37
Toxicodendron radicans (L.) Kuntze	14	0.37
	Total	100.13
TREES		
Juniperus virginiana L.	100	41.17
Cotinus obovatus Raf.	100	19.17
Diospyros virginiana L.	86	12.15
Bumelia lanuginosa (Michx.) Pers.	100	6.84
Rhamnus caroliniana Walt.	86	4.77
Celtis tenuifolia Nutt.	100	4.43
Fraxinus americana L.	71	3.67
Cornus drummondii C. Meyer	57	1.96
Cercis canadensis L.	43	1.54
Quercus prinoides Willd.	29	1.30
6 other species		3.09
	Total	100.09

teau. Understory species with the highest frequencies were *Sporobolus neglectus* (100%), *Carex crawei* Dewey (90%), *Schizachyrium scoparium* (80%), *Hedyotis nigricans* (75%), *Euphorbia corollata* Engelm. (60%), and *Rudbeckia missouriensis* (55%). The total number of juniper seedlings in the glade was 6694, and 99.7% of these were in the 0.46-m or less height class. Average number of juniper seedlings per m^2 was 0.84. Hall (1955: 177) stated, "The Glade is a 'prairie' association with *Andropogon scoparius* [= *S. scoparium*] and *Rudbeckia missouriensis* contributing most to its aspect and *Andropogon scoparius* and *Sporobolus neglectus* contributing most to cover." Hall concluded that this dolomite glade was an edaphic subclimax, as did Erickson et al. (1942) for the dolomite glades of the northeastern Ozarks in general.

The flora and qualitative community ecology of limestone and dolomite glades (as well as that of sandstone, chert, and igneous glades) of the Missouri Ozarks have been studied extensively by Nelson and Ladd (1982, 1983) and Nelson (1985). Nelson and Ladd (1982: 5) stated, "Characteristic dominant vascular plants of these [dolomite] glades include *Andropogon scoparius, Bouteloua curtipendula,* and *Sorghastrum nutans*"; and for limestone glades, "Dominant vascular plants on these glades are *Andropogon scoparius* and *Bouteloua curtipendula*" (Nelson & Ladd, 1982: 5). Nelson (1985) listed *A. scoparius* and *B. curtipendula* as the dominant plants on limestone glades and *A. scoparius, B. curtipendula,* and *Sporobolus heterolepis* as dominants on dolomite glades.

Ver Hoef et al. (1993) quantitatively sampled the vegetation of 32 glades on Eminence and Gasconade dolomites in southeastern Missouri. *Schizachyrium scoparium* clearly was the most important species in these rocky forest openings. This C_4 perennial grass had a constancy of 100% in glades on each of the two geologic formations, and it was in the highest cover class (4, average % cover = 31.6) in all 32

glades, i.e., 10–100% cover class, geometric mean = 31.6. Other important species on the dolomite glades were *Andropogon gerardii, Calamintha arkansana, Carex meadii, Coreopsis lanceolata* L., *Echinacea pallida, Fimbristylis puberula* (Michx.) M. Vahl var. *puberula, Hedyotis nigricans, Liatris aspera* Michx., *Liatris cylindracea* Michx., *Panicum virgatum, Rudbeckia missouriensis, Silphium terebinthinaceum* Jacq., *Smilax bona-nox* L., *Sorghastrum nutans, Sporobolus clandestinus* (Biehler) A. Hitchc., and *S. vaginiflorus.* The most important woody species on these glades were *Juniperus virginiana, Bumelia lanuginosa, Diospyros virginiana, Pinus echinata* Miller, *Quercus prinoides* Willd., *Q. stellata* Wangenh., and *Ulmus alata.*

Keeland (1978) quantitatively sampled the vegetation of 13 calcareous glades in the Ozarks of northwestern Arkansas. He recognized four general community types, which in an apparent successional sequence are: (1) grass and cedar; (2) cedar; (3) cedar-hardwood; and (4) hardwood. Keeland (1978: 12) described the grassland-cedar type as ". . . a grassy slope with a few scattered woody species." Further, it ". . . is the typical Ozark cedar glade as described by Hall (1955) and Kucera and Martin (1957) in Missouri and Hite (1959) in Arkansas" (Keeland 1978: 9). For the representative stand of this type described by Keeland, *Juniperus virginiana* and *Quercus stellata* were the most important overstory trees, *Rhus glabra* L. the most important shrub, and *Schizachyrium scoparium* the most important herb. Overstory tree basal area on this cedar glade was low, ca. 4.8m²/ha. *Schizachyrium scoparium* also was the most important species in limestone cedar glades quantitatively sampled by Logan (1992) in the Buffalo National River area in the Ozarks of northwestern Arkansas (Table 2).

Table 2. The most important species in 20 limestone glades studied by Logan (1992) in northwest Arkansas. Only species with a presence of at least 50% and/or an average abundance of at least 1.00 are included in the list. Percentage presence and average abundance values were calculated from data in Logan (1992).

Species	Average abundance value[a]	% presence
Schizachyrium scoparium (Michx.) Nash	4.60	95
Juniperus virginiana L.	2.60	70
Helianthus hirsutus Raf.	2.05	70
Andropogon gerardii Vit.	2.00	55
Quercus stellata Wangenh.	1.95	60
Ulmus alata Michx.	1.65	70
Quercus prinoides Willd.	1.40	60
Croton monanthogynus Michx.	1.35	60
Opuntia humifusa (Raf.) Raf.	1.25	60
Eupatorium altissimum L.	1.25	45
Coreopsis tinctoria Nutt.	1.15	30
Dichanthelium acuminatum (Sw.) Gould & Clark	1.15	40
Fraxinus americana L.	1.15	50
Ruellia humilis Nutt.	1.15	60
Lespedeza capitata Michx.	1.10	40
Sporobolus vaginiflorus (Torr. ex Gray) Wood	1.10	30
Dalea purpurea Vent.	1.10	55
Echinacea pallida (Nutt.) Nutt.	1.05	40
Euphorbia corollata Engelm.	1.00	55
Rhus aromatica Ait.	1.00	55
Desmanthus illinoensis (Michx.) MacMillan ex Robins. & Fern.	1.00	35
Bumelia lanuginosa (Michx.) Pers.	0.90	50
Celtis tenuifolia Nutt.	0.85	50

[a] Abundance scale from 1 to 5: 1 = rare (only one or two individuals present); 2 = occasional; 3 = frequent; 4 = common; and 5 = abundant (widespread with high cover, a dominant or near-dominant).

Skinner (1979) placed 0.01-m² and 0.1-m² quadrats around individual plants of the Ozark cedar glade endemic *Penstemon cobaea* Nutt. var. *purpureus* Pennell, *Centaurium texense* (Griseb.) Fern., and *Stenosiphon linifolius* (Nutt.) Heynh. to determine species associates (as well as other site characteristics) of these three rare plant species in limestone glades of the southwestern Missouri Ozarks. *Sporobolus neglectus* had the highest percent occurrence in quadrats of both sizes placed around *Penstemon* and *Centaurium,* and *Schizachyrium scoparium* had the highest percent occurrence in those of both sizes placed around *Stenosiphon,* which grows in deeper soil (15.7 ± 6.1 cm, mean ± SD) than *Penstemon* (10.9 ± 5.6 cm) or *Centaurium* (5.3 ± 2.8 cm). Percent occurrence of *S. scoparium* was considerably higher in the 0.1-m² than in the 0.01-m² quadrats: 22 vs. 5, 37 vs. < 5, and 59 vs. 16 for *Penstemon, Centaurium,* and *Stenosiphon,* respectively. The increase in percent occurrence of *S. neglectus* with increase in size of quadrats was less dramatic: 46 vs. 32, 58 vs. 33, and 11 vs. 10 for *Penstemon, Centaurium,* and *Stenosiphon,* respectively (Skinner, 1979).

In a dolomite glade in the Ozarks of southeastern Missouri, two contiguous 7 × 7-m sample plots within a whole-glade sample unit of 100 contiguous 7 × 7-m plots dominated by *Schizachyrium scoparium* and classified as "glades" (i.e., open glades) were divided into 200 70 × 70-cm plots (Ver Hoef et al., 1993). At this small scale of sampling, the glade was divided into rocky glade, shallow soil glade, glade, and deep soil glade zones. *Sporobolus*

vaginiflorus was the dominant species in the rocky glade and shallow soil glade zones, and *S. scoparium* in the glade and deep soil glade zones (Ver Hoef et al., 1993). However, in two other dolomite glades in southeastern Missouri sampled by Ver Hoef et al. (1993), as described above, *S. vaginiflorus* was not an important species.

Results of studies by Skinner (1979) and Ver Hoef et al. (1993) support a statement made by Kucera and Martin (1957: 290) in their study of glades in the Ozarks of southwestern Missouri: "*Andropogon scoparius* [= *Schizachyrium scoparium*] was the principal dominant; *Sporobolus neglectus* was generally frequent and locally abundant." Hall (1955), Hicks (1981), and Logan (1992) also found that either *Sporobolus neglectus* or *S. vaginiflorus* was a relatively important species in the Ozark glades they studied. Nelson (1985) listed *S. neglectus* as a characteristic plant of dolomite glades, but not of limestone glades, in the Ozarks of Missouri. Thus, both quantitative and qualitative studies show that annual *Sporobolus* species can be locally dominant within the larger prairie-like community on limestone and dolomite glades in the Ozarks. As such, cedar glades of the type described for the southeastern United States (Baskin & Baskin, 1999) occur in microhabitats within xeric limestone prairies of the Ozarks.

Schizachyrium scoparium also is the dominant plant in Ozark glades in terms of dry mass production. In the Ozarks of southwestern Missouri, total annual herbage production on glades ungrazed for 4 and 21 years was 312.2 and 241.5 g/m^2, respectively, according to a study by Buttery (1960). Production by *S. scoparium* on these two exclosures (the term used by Buttery to mean areas fenced off to prevent cattle grazing) was 175.2 and 112.3 g/m^2, respectively, and by *Sporobolus neglectus* 24.7 and 7.9 g/m^2, respectively. In the 4-year exclosure, production by *Andropogon gerardii* and *Sorghastrum nutans* was 52.2 and 34.8 g/m^2, respectively, and in the 21-year exclosure 29.2 and 74.7 g/m^2, respectively. Thus the C_4 perennial grasses *S. scoparium, A. gerardii,* and *S. nutans* produced 84% and 89% of the total herbage on the 4- and 21-year exclosures, respectively (Buttery, 1960). Regarding herbage production on grazed glades, Buttery (1960: 235) stated, "In 1956, as now, the glades outside the exclosures were producing about 400 pounds of oven dry herbage per acre [44.9g/m^2], mostly the less desirable baldgrass [*S. neglectus*] and black-eyed Susan (*Rudbeckia hirta* L.) with a scattering of the more desirable little bluestem (*Andropogon scoparius* Michx.) and Indiangrass [*S. nutans*]." Thus, annual *Sporobolus* can be the dominant grass on overgrazed Ozark glades.

The Midwest

In the Midwest, cedar or limestone glades have been reported in Wisconsin, Illinois, and Indiana. *Schizachyrium scoparium* was the most prominent ground-layer species in what Curtis (1959) called cedar glades in Wisconsin, with a presence of 100% and an average frequency of 38%. Other species in the ground layer with relatively high values for presence and average frequency were the C_3 herbaceous perennial forbs *Aquilegia canadensis* L., *Anemone cylindrica* A. Gray, *Dalea purpurea, Tradescantia ohioensis* Raf., *Euphorbia corollata, Arenaria stricta* Michx., *Solidago nemoralis* Dryander, *Antennaria neglecta* E. Greene, *Viola pedata* L., and *Scutellaria leonardii* Epling; the C_4 perennial grasses *Andropogon gerardii, Bouteloua hirsuta* Lagasca, and *Bouteloua curtipendula*; and the C_3 perennial grass *Koeleria pyramidata* (Lam.) P. Beauv. (Curtis, 1959: table XVI-10, appendix, p. 573). The most important tree species was *Juniperus virginiana* (Curtis, 1959: table XVI-11, appendix, p. 574).

Schizachyrium scoparium had a frequency of 80–100% in 30 of 32 limestone glades sampled by Kurz (1981) in Illinois. Other important grasses were the C_4 perennials *Bouteloua curtipendula, Sorghastrum nutans,* and *Sporobolus aspera* (Michx.) Kunth. The important forbs were the CAM leaf succulent *Agave virginica*; the C_3 perennials *Aster oblongifolius, Brickellia eupatorioides* (L.) Shinn., *Echinacea pallida, Euphorbia corollata, Hedyotis nigricans,* and *Physostegia virginiana* (L.) Benth.; and the C_3 summer annual *Croton monanthogynus* Michx. *Schizachyrium scoparium* also was the most important species in the limestone glades of southern Illinois studied by Heikens and Robertson (1995).

Limestone glades have been reported from five counties in southern Indiana (Aldrich et al., 1982; Bacone et al., 1983; Homoya, 1987; Maxwell, 1987), and they obviously are dominated by perennial prairie grasses. For example, referring to a limestone glade in Perry County, Bacone et al. (1983: 368) stated, "Indian grass (*Sorghastrum nutans*), big bluestem (*Andropogon gerardii*) and little bluestem (*A. scoparius*) are common grasses." Referring to two cedar glades in Clark County, Maxwell (1987: 413) stated, "Little bluestem dominates both glades as a xeric, bunch-grass surrounded by patches of rocky pavement."

The Ozarks and Midwest limestone and dolomite glades described above are similar vegetationally to

Table 3. Average percent frequency of native plant taxa in 147 1 × 1-m quadrats in five stands of xeric prairie vegetation on the Peebles dolomite in Adams County, Ohio. Only plants with a frequency of ≥20% are listed (calculated from data in Braun, 1928).

Species	% frequency
Schizachyrium scoparium (Michx.) Nash	79.4
Euphorbia corollata Engelm.	53.6
Blephilia ciliata (L.) Benth.	50.8
Lithospermum canescens (Michx.) Lehm.	45.2
Agave virginica (L.) Rose	43.2
Ruellia humilis (Nees) Lindau	41.8
Silphium trifoliatum L.	38.2
Bouteloua curtipendula (Michx.) Torr.	32.4
Thaspium sp.	32.0
Pycnanthemum flexuosum (Walt.) Britton, Sterns & Poggenb.	29.8
Senecio plattensis Nutt.	29.4
Sorghastrum nutans (L.) Nash	29.0
Comandra umbellata (L.) Nutt.	27.6
Helianthus hirsutus Raf.	27.6
Andropogon gerardii Vit.	26.2
Zizia aptera (A. Gray) Fern.	25.4
Fragaria vesca L.	25.2
Prunella vulgaris L.	23.4
Delphinium exaltatum Ait.	23.2
Lobelia spicata Lam.	22.8
Scutellaria parvula Michx.	21.4
Solidago nemoralis Ait.	20.0

what Braun (1928) called xeric prairies on Silurian Dolomite in Adams County in southern Ohio. Here, prairie grasses, primarily *Schizachyrium scoparium*, also are the dominant plants (Table 3). Interestingly, Braun pointed out that although there is some overlap in species composition between the xeric prairies of Adams County and the cedar glades of the Nashville Basin, the vegetation is different.

Neither annual species of *Sporobolus* nor of other grasses seem to be an important component of cedar or limestone glades that have been sampled quantitatively in Wisconsin (Curtis, 1959) or Illinois (Kurz, 1981; Heikens & Robertson, 1995). However, in one of the xeric prairies, Agave Ridge, on Silurian dolomite in Adams County, Ohio, sampled by Braun (1928), *S. vaginiflorus* had a frequency of 68% in 25 1-m² quadrats. The dominants of this prairie were *Schizachyrium scoparium* (100% frequency) and *Bouteloua curtipendula* (84% frequency). Braun referred to Agave Ridge and one of the four prairies she sampled on Peebles Dolomite as being more xerophytic than the other three. However, the average frequency of *S. vaginiflorus* on the five Peebles dolomite prairies was only 14.8%. Neither is *S. vaginiflorus* an important component of the vegetation of limestone glades in Indiana (Aldrich et al., 1982; Bacone et al., 1983; Homoya, 1987; Maxwell, 1987).

Woody Plant Invasion into Ozark and Midwest Glades

Martin (1955: 106) stated that ". . . even the thin-soiled areas [in the Ozarks of southwestern Missouri] are being invaded by eastern redcedar (*Juniperus virginiana*), winged elm (*Ulmus alata*), and associated drought-tolerant trees." Using 1938 and 1975 USDA aerial photographs showing limestone and dolomite glades in southwestern Missouri, Kimmel and Probasco (1980) found a dramatic decrease in glade area with 0–15% woody cover and a corresponding increase in glade area with 50–100% woody cover. Lowell and Astroth (1989) used USDA aerial photographs to study natural succession of glades to forest in the Hercules Glades Wilderness Area (southwestern Missouri) from 1938 to 1986. They found that "Even those areas most favorable for glades [Gasconade soil, 305–365 m (a.m.s.l.), south or southwest slopes] continue to convert to forest" (Lowell & Astroth, 1989: 78). The area most favorable for glades and occupied by glades decreased from about 285 ha to about 200 ha (30%) via conversions of glade land to forest (see fig. 3 in Lowell & Astroth, 1989). Using 1955, 1966, and 1984 aerial photographs, Ver Hoef et al. (1993) also showed that the sizes of dolomite glades in the Ozarks of southeastern Missouri had decreased due to woody plant invasion.

Kimmel and Probasco (1980) concluded that the most important reason for the increase in woody plant cover on open glades between 1938 and 1975 was that the U.S. Forest Service had not used fire to manage glade (cattle) range. Lowell and Astroth (1989: 78) thought that their study ". . . appears to support the theory held by some ecologists (USFS, pers. comm.) that the glades are not a naturally occurring ecotype[?] which can maintain themselves independent of fire and/or human intervention." Likewise, Ver Hoef et al. (1993) attributed woody plant invasion into calcareous glades in southeastern Missouri to fire suppression. Fire frequency was high in the Missouri Ozarks in presettlement times (Guyette & McGinnis, 1982), and fire probably played an important role in the origin and maintenance of limestone and dolomite glades in that area.

According to Curtis (1959), cedar glades in Wisconsin: (1) may have originated by invasion of redcedar into a dry prairie on sites protected from fire; and (2) would in time succeed to oak forest.

Heikens (1991) speculated that, in the absence of fire, the perennial grass-dominated limestone glades in southern Illinois would succeed to barrens ("savannas") and then to forest.

By studying aerial photographs of limestone glade areas in southern Indiana, Aldrich et al. (1982) and Bacone et al. (1983) determined that these openings in the forest are decreasing in size. Aldrich et al. (1982: 484) stated, "Aerial photographs document the continuing shrinkage of these glades [in Harrison County], as they were nearly double their present size in the 1940s." Bacone et al. (1983: 372) stated, "The aerial photographs show a remarkable decrease in size [of the forest openings in Perry County] in the last forty years due to encroachment of woody species."

Braun (1928: 425) thought the xeric prairies in Adams County, Ohio, ". . . antedate settlement by white man and are undoubtedly primary." However, using USDA aerial photographs taken in 1938, 1950, 1965, and 1971, Annala et al. (1983) and Annala and Kapustka (1983) found that prairie has succeeded (is succeeding) to forest. For example, prairie openings in the Lynx Prairie Preserve, where the soils are "shallow and poorly developed" (Annala et al., 1983: 22), covered 47% of the Preserve in 1938 but only 16% in 1971 (Annala et al., 1983; also see Foré & Guttman, 1996). Based on lack of difference in opal (phytolith) mass between soils presently under prairies and those presently under cedar-hardwood forests on dolomite in Adams County, Boettcher and Kalisz (1991: 127) concluded that ". . . the long-term vegetative history has been generally uniform over all areas on dolomite regardless of present occupancy by prairie or forest." Further, ". . . the distinction between primary and secondary prairies has little meaning since prairies only occur on areas of dolomite, and prairies and forest have alternated over time on these areas" (Boettcher & Kalisz, 1991: 127).

Concluding Remarks

The vegetation of limestone and dolomite glades in the Ozarks and Midwest is dominated by C_4 perennial prairie grasses (primarily *Schizachyrium scoparium*), and burning or some other method for removal of woody plant invaders is required to prevent succession to forest in these rocky, calcareous openings. Thus, they differ from cedar glades in the southeastern United States, in which the dominants are C_4 summer annual grasses (primarily *Sporobolus vaginiflorus*), and succession to forest does not occur in the absence of burning or other forms of management (Baskin & Baskin, 1999). The structure of the vegetation of the limestone and dolomite glades in the Ozarks and Midwest is much more similar to the xeric prairies on Silurian dolomite described by Braun (1928) in Adams County, Ohio, and to the xeric prairies on Mississippian limestone described by Baskin et al. (1994) on and adjacent to the Kentucky Karst Plain, than it is to what traditionally has been called cedar glades in the southeastern United States. Küchler's (1964) vegetation map of the United States shows the same vegetation type for cedar glades in the Ozarks and in middle Tennessee–northern Alabama. However, ample evidence is available in the literature to show that the glade vegetation of these two regions is quite different. Thus, we suggest that the rocky, calcareous, anthropogenic, prairie-grass-dominated openings in the Ozarks and Midwest be called xeric limestone prairies, in contrast to cedar glades, which are an edaphic climax dominated by C_4 summer annual grasses.

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STATISTICAL SUMMARY OF SOME OF THE ACTIVITIES IN THE MISSOURI BOTANICAL GARDEN HERBARIUM, 1999

	Vascular	Bryophyte	Total
Acquisition of specimens			
Staff collections	31,644	2,532	34,176
Purchase	21,173	0	21,173
Exchange	28,038	5,026	33,064
Gifts	5,575	1,219	6,794
Total acquisitions	86,430	8,777	95,207
Mountings			
Newly mounted	49,199	20,424	69,623
Mounted when received	21,173	0	21,173
Total specimens filed	70,372	20,424	90,796
Repairs			
Specimens repaired	32,134	n/a	32,134
Specimens stamped	1,585	n/a	1,585
Total repairs	33,719	0	33,719
Specimens sent			
On exchange	8,242	5,254	13,496
As gifts	11,212	637	11,849
Total	19,454	5,891	25,345
Loans sent			
Total transactions	375	46	421
Total specimens	32,876	2,959	35,835
To U.S. institutions			
Transactions	198	22	220
Specimens	16,810	1,408	18,218
To foreign institutions			
Transactions	177	24	201
Specimens	16,066	1,551	17,617
To student investigators			
Transactions	72	8	80
Specimens	11,244	967	12,211
To professional investigators			
Transactions	303	38	341
Specimens	21,632	1,992	23,624
Loans received			
Transactions	278	53	331
Specimens	32,303	4,062	36,365

The "Mounted when received" vascular plants are specimens of Chinese plants purchased directly from China.

From U.S.A.	From abroad	Total
581	507	1,088

The total number of mounted, accessioned specimens in the herbarium on 1 January 2000 was 5,002,128 (4,672,010 vascular plants and 330,118 bryophytes).

The Garden's herbarium is closely associated with its database management system, TROPICOS. The charts below summarize some of the statistics from TROPICOS both for the calendar year 1999 and as year-end totals. Note that the specimen records in TROPICOS are primarily based on MO specimens, meaning that about twenty-four percent of the bryophytes (an increase of about four percent over 1998) and twenty-nine percent of the vascular plants (an increase of about two percent) in the herbarium are now computerized, with an overall total of about twenty-eight percent (an increase of about one percent).

TROPICOS records—1999 additions

	Bryophytes	Vascular Plants	Total
Specimens	18,638	95,617	114,255
Names	1,290	25,016	26,306
Synonyms	2,135	18,828	20,963
Distributions	550	28,816	29,366
Types	63	18,660	18,723
Bibliography	1,343	2,333	3,676

TROPICOS records—Year-End 1999 Totals

	Bryophytes	Vascular Plants	Total
Specimens	79,489	1,339,708	1,419,197
Names	97,184	747,614	844,798
Synonyms	61,084	370,118	431,202
Distributions	36,991	740,385	777,376
Types	6,826	245,157	251,983
Bibliography	20,606	58,025	78,631
Specimens in herbarium	330,118	4,672,010	5,002,128
Percent computerized	24	29	28

In TROPICOS, literature-based Synonymy is always linked to a reference in Bibliography and directly with at least two records in Names, the synonym, usually a basionym, and the correct name. Additional synonymy may be derived from these direct links, e.g., all other combinations of a basionym treated as a synonym of a given name are also synonyms of it. With the completion of *A Checklist of the Mosses* (www.mobot.org/mobot/tropicos/most), which required recording synonymy and acceptance of the 58,000 valid names of moss species (about 13,000 species are recognized), the filing of the mosses in the Bryophyte Herbarium will be adjusted to this new standard.

—Marshall R. Crosby

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Orchids

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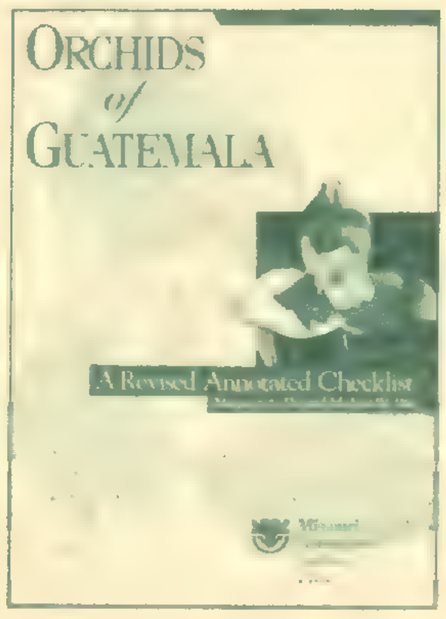

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Cover illustration. *Cratera simplicifolia* J. S. Miller, drawn by Barbara Alongi, from the booklet for the 15th Annual Systematics Symposium, Missouri Botanical Garden. First published in *Novon* 8: 167–169 (1998).

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THE REDISCOVERY OF A MALAGASY ENDEMIC: *TAKHTAJANIA PERRIERI* (WINTERACEAE)[1]

George E. Schatz[2]

ABSTRACT

The history and rediscovery of *Takhtajania perrieri* (Capuron) Baranova & J.-F. Leroy, the only extant representative of the primitive family Winteraceae in the Africa/Madagascar region, is recounted.

Key words: discovery, Madagascar, *Takhtajania*, Winteraceae.

For most of the 20th century, *Takhtajania perrieri* (Capuron) Baranova & J.-F. Leroy stood as the Holy Grail of Malagasy botany. Numerous expeditions searched in vain for the only possible living member of the ancient family Winteraceae in the Africa–Madagascar region at the Manongarivo Special Reserve in northwestern Madagascar, where, in 1909, Henri Perrier de la Bâthie collected the only known specimens. With hundreds of square kilometers of intact forest protected within the Manongarivo reserve, the species must surely still exist there! Nevertheless, the dozens of botanists who ascended the slopes of Mt. Antsatrotro to the 1700-meter elevation cited on Perrier de la Bâthie's handwritten labels, passing through what is infamously acknowledged to be the most dense zone of terrestrial leeches in all of Madagascar, have all failed to relocate *Takhtajania*.

By the spring of 1997, I commenced work on the *Generic Tree Flora of Madagascar* (Schatz, in press). The *Tree Flora* will serve as a revision and expansion of René Capuron's 1957 *Essai d'Introduction à l'Etude de la Flore Forestière de Madagascar*, a work existing only in mimeographed

[1] At the Missouri Botanical Garden, I thank P. H. Raven and P. P. Lowry II for the opportunity to study the Malagasy flora, and V. Hollowell and P. P. Lowry II for helpful comments on the manuscript. In Paris, Ph. Morat and his staff have always extended the most cordial hospitality during my visits to the Laboratoire de Phanérogamie. In Madagascar, fieldwork was conducted under collaborative agreements between the Missouri Botanical Garden and the Parc Botanique et Zoologique de Tsimbazaza and the Direction de la Recherche Forestière et Piscicole, FOFIFA, Antananarivo, Madagascar. Fieldwork would have been impossible without the assistance of the World Wide Fund for Nature; special thanks go to Jean Marc Garreau and Désiré Ravelonarivo at their Andapa office. I gratefully acknowledge courtesies extended by the Government of Madagascar (Direction Générale de la Gestion des Ressources Forestières) and by the Association Nationale pour la Gestion des Aires Protégées. This research was conducted with support from U.S. National Science Foundation grants DEB-9024749 and DEB-9627072, and grants from the National Tropical Botanical Garden, Kauai, and the National Geographic Society.

[2] Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166-0299, U.S.A.

form. Although it would be another six years before he described *Bubbia perrieri* [= *Takhtajania perrieri*] (Capuron, 1963), by 1957 Capuron had already determined that *Perrier de la Bâthie 10158* constituted the first and only record of the family Winteraceae in Madagascar, including a description of the family in his *Essai,* as represented in Madagascar by "Un seul genre et une seule espèce: Bubbia Perrieri R. Cap." (p. 96), with the further note that "La famille est nouvelle pour Madagascar; l'espèce provient du massif du Manongarivo" (p. 104). In Paris during March of 1997, I decided to write the description of *Takhtajania* for the *Tree Flora,* and, that to do so, it was high time that I examine the type specimen. After several inquiries, two sheets of *Perrier de la Bâthie 10158* were located in the office of Thierry Deroin. He, with the late Jean-François Leroy (1915–1999), had recently studied the vasculature of the ovary utilizing the type material (Deroin & Leroy, 1993). Over the course of several days as I wrote my generic description, I stared periodically at those enigmatic specimens collected nearly 90 years earlier. While wondering whether the species might still exist, I had to conclude my treatment of the genus with the unsatisfactory statement that despite numerous attempts, *Takhtajania* had not been seen again. Two months later, back in St. Louis, with the images of those type specimens still very fresh in my mind's eye, I opened the enfolding newspapers of a collection tentatively determined as "Rhamnaceae," made in 1994 from the Anjanaharibe-Sud Special Reserve by the Malagasy collector Fanja Rasoavimbahoaka, and, eureka, there it was! In an instant, I realized that *Takhtajania* had indeed been seen again, but not where we had been looking for it. Set into motion that momentous day were a series of events culminating in the following papers.

From "Indet." To Political Cartoon: One Species' History

The first documented human contact with *Takhtajania* occurred in May 1909 on the Manongarivo Massif in northwestern Madagascar, a chance event preserved for all time as two herbarium specimens (*Perrier de la Bâthie 10158*) deposited at the Muséum Nationale d'Histoire Naturelle in Paris. Both Capuron (1963) and Leroy (1978, 1993) transcribed the locality data on Perrier de la Bâthie's handwritten label as "Massif du Manongarivo, au bord des ruisseaux, sur schistes liasiques, vers 1,700 m d'altitude." It seems likely, however, that Perrier de la Bâthie's altimeter was inaccurate, insofar as he attributed some of his collections from the same trip to 2000 m, despite a maximum altitude for the massif of 1876 m at the summit of Mt. Antsatrotro. Given this, and that *Takhtajania* is now known to range from 1100 to 1550 m altitude at its second known locality within the Anjanaharibe-Sud Special Reserve, this may partially explain why it has never been relocated at Manongarivo: we may well have been looking for it at too high an elevation! Accompanying the two type sheets enclosed within a red Type folder are Perrier de la Bâthie's field notes. These were reproduced in full by Leroy (1978), and reveal that *Takhtajania* first entered our collective botanical consciousness as a "family indet."; based upon its leaf, stamen, and ovary characteristics, Perrier suggested both "Annonaceae?" and "Dilleniaceae? (*Tetracera*)," believing the single carpel to correspond to a carpel of a flower with multiple, free carpels. A later annotation (with a preprinted partial date of "193 ," the specific year left blank) affixed to one of the sheets by J. Ghesquière, a specialist in Annonaceae, rejected it from that family by noting simply "Magnol.," perhaps as an ordinal placement. Leroy (1978) cited undated notes referring to the winteraceous genera *Bubbia* and *Drimys* by H. Humbert; although he may have been the first to recognize the correct family placement, these notes no longer accompany the type specimens. Nevertheless, *Takhtajania* remained nameless for 54 years until Capuron (1963) formally described it as *Bubbia perrieri.* Such a lag time between field collection and its actual description serves as a useful illustration of the occasionally slow pace of botanical studies.

Having finally received a name, *Bubbia perrieri* soon became the focus of additional studies on pollen (Straka, 1963; Lobreau-Callen, 1977) and leaf anatomy (Baranova, 1972; Bongers, 1973). These studies reported colpate or trichotomocolpate (trichotomosulcate) pollen apertures and an anomocytic stomatal cell arrangement, both features anomalous within the Winteraceae (neither condition is corroborated herein by Keating or Sampson, respectively). Stimulated by these novel findings, Leroy (1977) expanded upon Capuron's observation of a bilobed stigma for *Bubbia perrieri,* and daringly hypothesized a compound unilocular ovary comprised of two united carpels; he also presciently suggested a close alliance to Canellaceae. For Leroy (1978), such radical differences clearly merited distinct higher-taxon status, and thus a new genus was described. *Bubbia perrieri* Capuron became *Takhtajania perrieri* (Capuron) Baranova & J.-F. Leroy, and the new genus became the type of a wholly new subfamily Takhtajanioideae. When

Vink's (1978) studies of the ovary concurred with his bicarpellate hypothesis, Leroy felt sufficiently vindicated against Tucker and Sampson's (1979) cautionary skepticism in the journal *Science* that his enthusiasm knew no bounds. In 1980, he raised his subfamily Takhtajanioideae to the rank of family: Takhtajaniaceae, indeed, from a "family indet." to a family apart!

Meanwhile, numerous botanists failed to relocate *Takhtajania* at the Manongarivo Special Reserve. Capuron collected there extensively in 1954, including the upper slopes of Mt. Antsatrotro, but then he apparently never returned. In 1966, he stayed closer to the main road from Maromandia to Ambanja that passes ca. 10 km to the west of the Reserve boundary, and fully 25 km west of the upper slopes of Antsatrotro. Vink (1978) recounted that Capuron told him that the type locality was completely deforested, a curious remark given the vast area of intact forest present still today (Gautier et al., 1999). Several years later, Josef Bogner (M) searched for Araceae on the southern slopes of Antsatrotro north of Bejofo, and the late Al Gentry walked in from the road to as high as 300 m altitude in 1974. Then, at the behest of Leroy, Raymond Rabevohitra (TEF) once again searched the upper slopes of Antsatrotro in 1979, but found no trace of *Takhtajania*. Beginning in May of 1989 (Schatz, 1989), the Missouri Botanical Garden has conducted numerous expeditions to Manongarivo, and since 1994, Laurent Gautier (1997, 1999) has headed Geneva's ongoing inventory efforts there. For all who made the arduous pilgrimage to Manongarivo, *Takhtajania* would remain elusive.

Some 150 km to the southeast of Manongarivo, also in 1994, Fanja Rasoavimbahoaka began work as a local collector at the Marojejy Strict Nature Reserve (now a national park) and Anjanaharibe-Sud Special Reserve in conjunction with an AN GAP (the National Association for the Management of Protected Areas)/World Wide Fund for Nature protected areas project. That May, he ascended a trail used by local gem and crystal hunters leading west from the abandoned village of Andranotsarabe, which had served as a camp, gravel depository, and water source during the building of the road connecting Andapa and Bealanana that bisects the Anjanaharibe-Sud Reserve. Unbeknownst to him, he collected *Takhtajania* in flower (Fig. 1; additional images of *Takhtajania* may be found on the web at http://www.mobot.org/MOBOT/Madagasc/winterac.html), exactly 85 years to the month after Perrier de la Bâthie's original collection from Manongarivo. Later that same year, a multi-disciplinary study of altitudinal variation, sponsored by WWF and headed by Steve Goodman (1998), used this same trail to access the summit of Anjanaharibe-Sud. In fact, they established their 1200-m "Camp 2" right amidst the *Takhtajania* population. Expedition botanist Désiré Ravelonarivo collected fruiting *Takhtajania* both in September and November, and all of the expedition members must have walked by *Takhtajania* numerous times along the trail.

This brings us forward to that fateful day in May 1997, when I opened the newspapers containing *F. Rasoavimbahoaka 303*. The first person to whom I showed the collection was Peter H. Raven, who had launched MBG's activities in Madagascar several decades earlier, partly in hopes of finding *Takhtajania* again. He suggested that I immediately prepare an announcement of the rediscovery for the journal *Nature* (Schatz et al., 1998). I then dispatched e-mails to Paris and Madagascar, sounding the call to action. Several weeks later, members of our Malagasy staff including Sylvie Andriambololonera, Jeannie Raharimampionona, and Pierre Jules ("Coca") Rakotomalaza accompanied Désiré back to the *Takhtajania* locality at Anjanaharibe-Sud, photographing its flowers and collecting the first lot of specialized research materials that would be utilized in the following new studies. The following month, a specimen for the Komorov Institute in St. Petersburg was presented to Armen Takhtajan at a special birthday celebration for him in St. Louis; born just one year after Perrier de la Bâthie first collected *Takhtajania*, he had waited 87 years for its rediscovery.

Since then, *Takhtajania* has been revisited numerous times. The journey from Andapa to Andranotsarabe by 4-wheel-drive vehicle takes three hours, if it has been relatively dry the previous days. However, subjected to over 3600 mm of rainfall per year, portions of the road are deteriorating rapidly, and landslides threaten continued access to the population. Having arrived at the trailhead, one must gently climb little more than 200 m in elevation through beautiful forest. Even when it is raining—which it often is—the leech population does not even begin to compare with that of the slopes of Mt. Antsatrotro at Manongarivo. In less than an hour, one enters *Takhtajania*'s realm, the narrow, flattened ridges followed by the trail, and the uppermost portions of the steep slopes falling away on either side of the ridges. With its large, glossy, dark green leaves clustered toward the apex of vertical stems, *Takhtajania* stands out in the understory, its slender trunk often leaning severely after surviving the impact of fallen debris. Extending over eleven square kilometers, this second recorded *Takhtajania* population appears to be thriv-

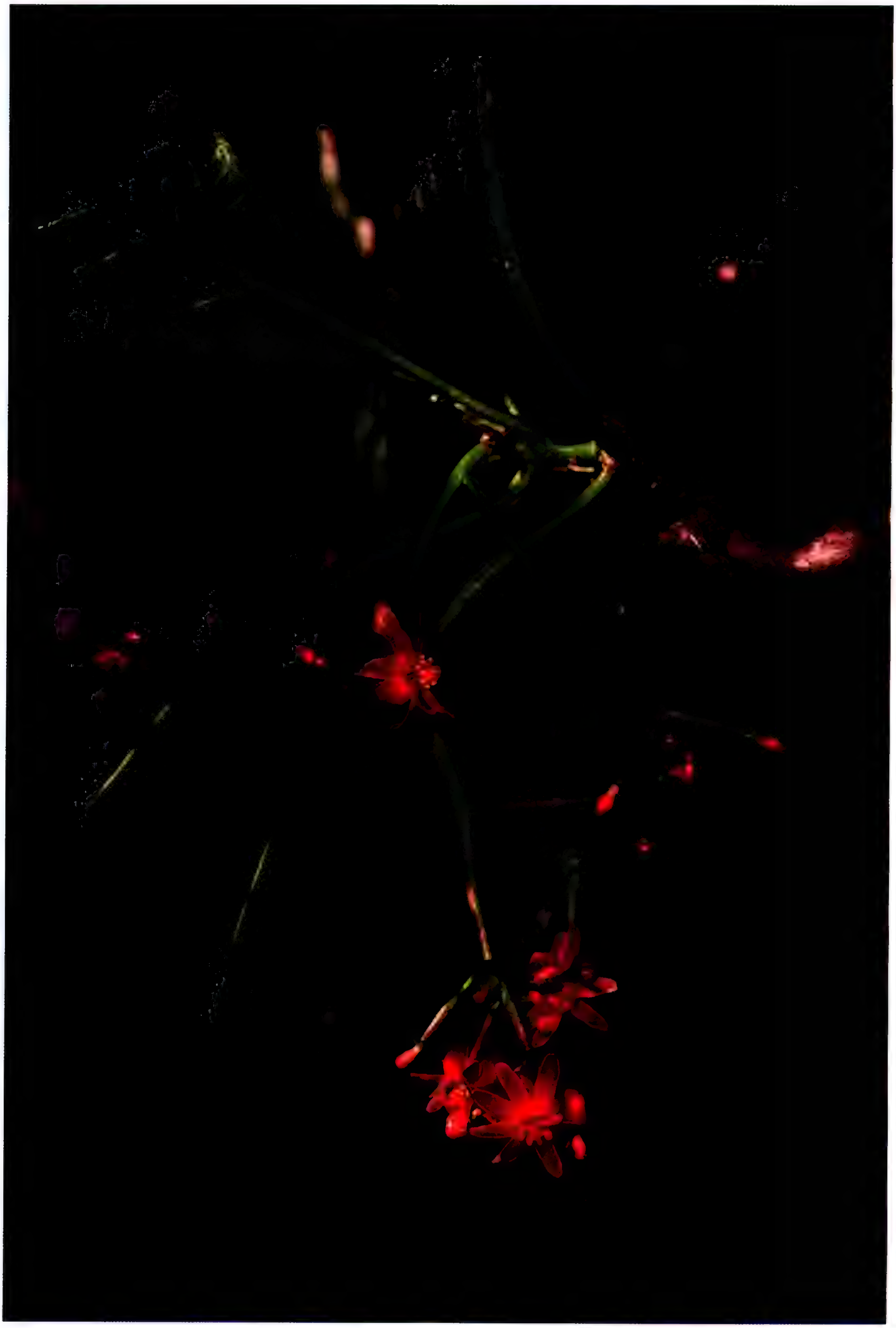

Figure 1. Flowering branch of *Takhtajania perrieri* (Capuron) Baranova & J.-F. Leroy at the Anjanaharibe-Sud Special Reserve, Madagascar.

ing; Birkinshaw and colleagues (1999a) have estimated a minimum of 42,020 individuals representing all size classes, including abundant seedlings.

In his 1998 New Year's address to representatives of the diplomatic community, the President of Madagascar, Didier Ratsiraka, heralded *Takhtajania* as a national treasure that dated back to the time of the dinosaurs. His remarks immediately drew the attention of other national figures. Ministers, at that time, of the Environment, and Waters and Forests, C. Vaohita and R. Rajonhson, respectively, accompanied by the Directors of Biodiversity Valorization, and Ecotourism at ANGAP, M. Faramalala and J. Rakotoarisoa, respectively, all made the long trek to see *Takhtajania.* Shortly thereafter, a political cartoon appeared in one of the daily Antananarivo newspapers depicting a Malagasy villager exclaiming deliriously his good fortune in the first panel: "Je suis riche! Riche! Riche! . . . J'ai . . . J'ai . . ." ("I am rich! Rich! Rich! . . . I . . . I . . ."), and then in the second panel, holding a potted seedling identified as *Takhtajania perrieri,* "J'ai faim . . ." ("I am hungry"). Ironically, *Takhtajania* holds absolutely no value whatsoever for the villagers living in the immediate area. When shown the plant, they had neither a use nor a vernacular name for it. Nevertheless, *Takhtajania* continues to shine prominently on the political landscape, having been chosen as the emblem for Madagascar's exhibit at EXPO-2000 in Hanover, Germany (during the summer of 2000), highlighting the government's conservation and sustainable development programs.

Rediscovery of *Takhtajania* has provided wonderful new research opportunities, the first results of which are reported in the following collection of papers. James Doyle sets the stage by reviewing the fossil history of Winteraceae. Sherwin Carlquist confirms the vesselless nature of *Takhtajania* wood, whereas Taylor Feild and his colleagues examine the condition of vesselless wood from an ecophysiological perspective. Reexamining foliar anatomy, Richard Keating reports mostly brachyparacytic stomata, and thus discounts the original reports of an anomocytic arrangement that contributed to the initial justification of generic status for *Takhtajania*; furthermore, he summarizes a unique nodal anatomy in *Takhtajania.* Peter Endress and his colleagues detail its floral morphology, by virtue of which *Takhtajania* conforms most closely to *Pseudowintera* and *Zygogynum,* in part, due to its small, early-rupturing involucre and apical anther sacs. It was these two features that induced Capuron (1963) to describe the species originally in the genus *Bubbia,* which is now included by some authors in *Zygogynum* s.l. Further exploring the flowers, Andrew Doust examines the ontogeny of perianth parts in Winteraceae. His comparative sequential data suggest that the similarities of an early-rupturing involucre and perianth arrangement exhibited by *Takhtajania* and *Zygogynum* reflect the plesiomorphic state, and that petal fusion in the two genera is non-homologous. His results further imply that the enveloping late-rupturing involucre of *Tasmannia* and *Drimys* has evolved independently in each genus. Delving deeper into the flower, Bruce Sampson reports round or slightly oval apertures in pollen of *Takhtajania,* thus dispelling earlier reports of colpate and trichotomocolpate (trichotomosulcate) apertures; he also finds possible evidence for asynchronous pollen mitosis within a tetrad similar to that in *Drimys* and *Pseudowintera.* Hiroshi Tobe and Bruce Sampson report embryological features indistinct from other Winteraceae, but well distinguished from probable sister Canellaceae. Thierry Deroin's examination of fruit vascular anatomy reveals that the nutritive role is transferred from the median carpellary bundle to the lateral bundles in maturing ovaries during fruit development. At the cytological level, Friedrich Ehrendorfer and M. Lambrou document a "diploidized," possibly paleotetraploid, chromosome number of $2n = 36$, which can only be understood in relation to the paleopolyploidy exhibited by other Winteraceae ($n = 13$ to $n = 43$) by presuming numerous extinction gaps. An analysis of molecular sequence data by Ken Karol and colleagues provides phylogenetic context, firmly placing *Takhtajania* basal and sister to remaining Winteraceae, a result consistent with Leroy's subfamilial designation. Reported elsewhere are studies of the habitat of *Takhtajania* (Birkinshaw et al., 1999b), and an assessment of its risk of extinction (Birkinshaw et al., 1999a). Later this year, Rivolala Andriamparany concludes his studies of the reproductive biology of *Takhtajania perrieri* at the University of Antananarivo under the direction of Elizabeth Rabakonandriana and Len Thien of Tulane University.

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Note added in proof. It happened again! On the very day all of the manuscripts were sent to Allen Press, Madagascar Program coordinator Kendra Sikes and I were looking through backlogged local collector specimens still in their original newspapers collected on the Masoala Peninsula in January, 1996, by Jao Aridy. There was *Takhtajania* in fruit! As before, I immediately dispatched E-mail alert to our Madagascar office, as well as to the Wildlife Conservation Society, who oversees the new Masoala National Park. A successful mission to relocate this new population of *Takhtajania*, which included original collector Jao Aridy and Jean Fortunat Toto of ANGAP/WCS, Rivolala Andriamparany of the University of Antananarivo, and Johny Rabenantoandro of MBG, has now returned from the field. During four days of arduous, wet searching, they found only three plants of *Takhtajania*—one sparsely flowering adult, one sterile adult, and one seedling—located ca. 6 km northeast of Ambanizana (15°34′07″S, 50°00′14″E, or ca. 110 km south of the Anjanaharibe-Sud Special Reserve population) at 790 m altitude on the western slopes of Mt. Ambohitsitondroina. Rivolala Andriamparany, the only member of the team familiar with the Anjanaharibe-Sud population, reports that the flowers of the new population are similar to those he has studied at Anjanaharibe-Sud, but that the leaves and overall habit (smaller, shrubbier) appear somewhat different, confirming my initial impression from the original Jao Aridy collection that the leaves are less thick and coriaceous, and moreover, lack a revolute margin. Along with the significantly lower elevation (790 m vs. 1100–1500 m at Anjanaharibe-Sud), the potential morphological differences tantalizingly suggest the possibility of a second, distinct taxon within *Takhtajania*!

PALEOBOTANY, RELATIONSHIPS, AND GEOGRAPHIC HISTORY OF WINTERACEAE[1]

James A. Doyle[2]

ABSTRACT

In combination with phylogenetic analyses of morphological and molecular data from modern plants, fossils allow improved reconstruction of the history of Winteraceae. Phylogenetic analyses link Winteraceae with Canellaceae, nested among Magnoliales, Laurales, and Piperales, implying that their lack of vessels is derived rather than primitive. The oldest records, from the Barremian and Aptian-Albian (Early Cretaceous) of Northern Gondwana (Gabon, Israel), are ulcerate tetrads (*Walkeripollis*) with an annulus underlain by thickened endexine but finer sculpture than modern Winteraceae, which most likely represent the stem-lineage leading to crown-group Winteraceae. Early Cretaceous *Afropollis* and *Schrankipollis*, which have also been compared with Winteraceae, are probably not related. The distribution of *Walkeripollis* implies that the winteraceous line originated in tropical, possibly dry, environments, like those of modern Canellaceae, rather than equable temperate and upland tropical habitats like those where Winteraceae occur today. Crown-group Winteraceae, first represented in the Santonian-Campanian (Late Cretaceous) by more coarsely reticulate tetrads in Australia and vesselless wood in Antarctica, and later by tetrads in South America and Africa, appear to be derived from a line that spread south into the temperate zone, consistent with ecological hypotheses that vessels were lost as an adaptation to cooler climates. Late Cretaceous paleogeography suggests that the crown-group could have dispersed to Australasia via either South America and Antarctica or Madagascar (where the family is represented by the basal genus *Takhtajania*) and India.

Key words: angiosperms, biogeography, Cretaceous, paleobotany, palynology, phylogeny, Tertiary, Winteraceae.

Winteraceae have long attracted students of angiosperm evolution and biogeography because of their putatively primitive morphology and disjunct Southern Hemisphere distribution (Australasia, South America, Madagascar). Emphasizing their vesselless wood and plicate (conduplicate) carpels, Thorne (1974) considered Winteraceae the most primitive living angiosperm family. Others (Walker & Walker, 1984; Cronquist, 1988; Takhtajan, 1997) argued that Magnoliales such as *Degeneria* are more primitive: although these have vessels, they also have plicate carpels, plus gymnosperm-like monosulcate pollen with a continuous tectum and granular infratectal structure, whereas Winteraceae have putatively more advanced pollen shed in permanent tetrads, with a round distal pore (ulcus), coarsely reticulate sculpture, and columellar infratectal structure. After concluding that angiosperms arose in the area of Southeast Asia and Australasia (cf. Takhtajan, 1969), Smith (1973) postulated that Winteraceae originated in Malesia and migrated south through Australasia and Antarctica to South America and west to Madagascar. This scenario was challenged by the theory of plate tectonics and recognition that Southeast Asia and Australasia are juxtaposed portions of two supercontinents, Laurasia and Gondwana, which were widely separated during the rise of angiosperms in the Early Cretaceous. Since only two species of Winteraceae occur outside the former limits of Gondwana (*Drimys granadensis* L. f. in Central America, *Tasmannia piperita* (Hook. f.) Miers in the Philippines), which could be "spillovers" after South America and Australasia collided with Laurasia in the Tertiary, Winteraceae were soon offered as one of the best examples of a taxon with a Gondwanan distribution (Schuster, 1972, 1976; Raven & Axelrod, 1974).

Paleobotanical discoveries and phylogenetic analyses of the past two decades have shed new light on the history of Winteraceae and other angiosperm taxa. The purpose of this paper is to review the fossil record of Winteraceae in the context of the phylogenetic results, and thus to present an updated synthesis of the evolutionary and geographic history of the family, as background for discussion of *Takhtajania*. Of special interest is fossil pollen evidence extending the winteraceous line into the Early Cretaceous, which indicates that

[1] I am grateful to Rosemary Askin and Mary Dettmann for help in locating literature, Sharma Gaponoff, Jean-Michel Groult, Bob Hill, Sarah Mathews, Imogen Poole, Hervé Sauquet, Vincent Savolainen, George Schatz, Alex Troitsky, and Liz Zimmer for use of unpublished data, Neelima Sinha for help with illustrations, and Else Marie Friis and an anonymous reviewer for useful comments on the manuscript.

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Winteraceae originated in more tropical environments than they inhabit today.

Phylogenetic Context

Phylogenetic analyses have confirmed the position of Winteraceae among magnoliids, now recognized as a basal paraphyletic grade of angiosperms below monocots and eudicots (the 95% of dicots with tricolpate and derived pollen). However, they call into question the primitive status of vesselless wood, at least in Winteraceae.

Based on a morphological cladistic analysis of primitive angiosperms, Young (1981) concluded that Winteraceae and other vesselless taxa are nested well within the angiosperms, so it is more parsimonious to assume that vessels arose in the common ancestor of angiosperms and were lost in the vesselless taxa. This was confirmed by the analysis of Donoghue and Doyle (1989), which placed Magnoliales in a restricted sense, with granular exine structure, at the base of the angiosperms, with the remaining groups (including Winteraceae) united by columellar structure. Winteraceae were linked with Illiciales (*Illicium*, Schisandraceae) and in some trees Canellaceae, based on palisade exotesta, a similarity of Winteraceae, Illiciales, and Canellaceae noted by Corner (1976). Winteraceae and Illiciales had also been associated by Walker (1976), based in part on their coarsely reticulate pollen sculpture, and were subsequently linked in the morphological cladistic analysis of Loconte and Stevenson (1991). Another similarity of Winteraceae and Canellaceae is their irregular, "first rank" leaf venation (Hickey, 1977), with secondary veins that are poorly differentiated from higher vein orders and loop repeatedly inside the margin (Hickey & Wolfe, 1975). However, this may be a symplesiomorphy rather than evidence for direct relationship; similar venation occurs in Early Cretaceous angiosperm leaves and has been considered primitive (Wolfe et al., 1975; Doyle & Hickey, 1976; Hickey, 1977).

Since vessels are often viewed as unambiguously advantageous in allowing more efficient conduction, these cladistic results stimulated debate on the functional plausibility of loss vs. multiple origins of vessels. Donoghue and Doyle (1989) argued that loss might be less deleterious than assumed, since vesselless wood would be derived from wood with very primitive vessels, not advanced ones. Winteraceae and other vesselless angiosperms usually occur in cool, wet habitats; Carlquist (1975) argued that this is the only environment where they can persist, but Donoghue and Doyle (1989) noted that it might also be the environment where loss of vessels would be least disadvantageous. Donoghue and Doyle and Feild et al. (2000) suggested that vessel loss might even be advantageous in cool climates, since embolisms caused by freezing would be restricted to one tracheid rather than expanding to fill a whole vessel. This is especially plausible for Trochodendrales (nested among lower eudicots with vessels: Chase et al., 1993; Hoot et al., 1999; Savolainen et al., 2000), which were common in the Late Cretaceous and Tertiary of northern Laurasia (Crane et al., 1991). Carlquist (1983, 1987, 1988, 1996) presented several arguments against the loss of vessels and for multiple origins: the parallel nature of advancement trends within vessels; the implausibility of scenarios for loss of vessels as a result of movement from dry to mesic (Young, 1981) or from aquatic to terrestrial habitats; the fact that vessels are almost but not completely lost in *Ephedra*, even in extreme habitats; and the lack of tracheid dimorphism in vesselless angiosperms. However, one argument of Bailey and Nast (1944) and Carlquist (1975), that the characteristic stomatal plugs of Winteraceae are a compensation for lack of vessels, appears to be incorrect. Feild et al. (1998, 2000) showed experimentally that plugs do not reduce transpiration and are more likely a device to promote runoff of water in cloud forest habitats.

Many aspects of earlier cladistic schemes now need revision as a result of molecular phylogenetic analyses. Studies of partial rRNA sequences (Hamby & Zimmer, 1992; Doyle et al., 1994) rooted angiosperms not in woody magnoliids but in "paleoherbs," with Nymphaeales basal, as did more recent morphological analyses (Doyle et al., 1994; Doyle, 1996). Much more extensive studies of *rbc*L (Chase et al., 1993) placed *Ceratophyllum* at the base of the angiosperms. However, this rooting was quickly suspected to be a long-branch effect (Qiu et al., 1993; Donoghue, 1994). This view has been confirmed by analyses of 18S rDNA (Soltis et al., 1997), cpITS (Goremykin et al., 1996; A. V. Troitsky, pers. comm. 1998), phytochrome genes (Mathews & Donoghue, 1999), and *atp*B (Savolainen et al., 2000), all of which root angiosperms among a series of taxa including not only Nymphaeales but also several woody magnoliid taxa: *Amborella*, *Austrobaileya*, Illiciales, and (based on studies of *rbc*L by Renner, 1999) Trimeniaceae. Significantly, these taxa occur together as a clade in *rbc*L trees (Chase et al., 1993). Endress (1987) and Donoghue and Doyle (1989) associated *Amborella* and Trimeniaceae with Chloranthaceae, but Chloranthaceae

now appear to be an isolated line, located above the basal grade.

All molecular analyses that have included the relevant taxa have separated Winteraceae from Illiciales and linked them with Canellaceae (Chase et al., 1993; Soltis et al., 1997; Mathews & Donoghue, 1999; Savolainen et al., 2000), making up a group that I will call Winterales. All genes except 18S (Soltis et al., 1997), for which the sampling of magnoliids was less complete, place Winterales in a clade with three other orders as defined by APG (1998): Laurales (Calycanthaceae, Monimiaceae s.l., *Gomortega*, Hernandiaceae, Lauraceae), Magnoliales (*Eupomatia*, Himantandraceae, *Degeneria*, Myristicaceae, Magnoliaceae, Annonaceae), and Piperales (Piperaceae, Saururaceae, Aristolochiaceae, *Lactoris*). Based on *rbc*L (Chase et al., 1993) and *rbc*L plus *atp*B (Savolainen et al., 2000), the sister group of Winterales is Magnoliales; based on phytochrome genes (Mathews & Donoghue, 1999) and *atp*B (Savolainen et al., 2000), their sister group is Piperales.

Like the morphological trees, the molecular trees imply that Winteraceae are secondarily vesselless, although interestingly they suggest that the absence of vessels in *Amborella* is primitive. They also call into question the view that the carpels of Winteraceae are primitive: the basal lines (*Amborella*, *Austrobaileya*, etc.) have ascidiate, not plicate carpels (Endress & Igersheim, 1997), implying that the plicate condition is derived. Igersheim and Endress (1997) also refuted statements that the carpels of Winteraceae and *Degeneria* are not completely closed (Bailey & Swamy, 1951; Eames, 1961); actually, their margins are postgenitally fused.

These molecular challenges to morphological cladistic results appear to be supported by preliminary analyses of an expanded morphological data set of magnoliids and basal monocots and eudicots (Doyle & Endress, in prep.), incorporating gynoecial data of Endress and Igersheim (1997, 1999) and Igersheim and Endress (1997, 1998). These analyses also move Illiciales near *Austrobaileya* and associate Winteraceae with Canellaceae, based on palisade exotesta and truncate stamen connective.

Phylogenetic analyses have also led to explicit hypotheses on relationships within Winteraceae. Vink (1988) presented two morphological trees of the family, which differed in rooting: one with *Tasmannia* basal, on the standard assumption that the ancestral chromosome number is $n = 13$ (as in *Tasmannia*) and $n = 43$ (as in other members) is derived; the other with *Takhtajania* basal, based on its elongate inflorescences, and *Tasmannia* and *Drimys* linked as a clade, which he preferred. Because Vink assumed that *Takhtajania* has $n = 43$, based on its large pollen size (Praglowski, 1979), he suggested that $n = 13$ in *Tasmannia* is not ancestral but derived from $n = 43$. This poses a problem if the closest outgroup is Canellaceae, which have $n = 11$, 13, and 14 (Kubitzki, 1993). In both trees, *Bubbia*, *Belliolum*, *Zygogynum*, and *Exospermum* formed a clade, linked with *Pseudowintera*. Two species of *Zygogynum* (*Z. balansae* Tiegh., *Z. pomiferum* Baill.) differ from the rest of the family in having monad pollen grains (Sampson, 1974); the position of *Zygogynum* within the family implies that these are secondarily derived from tetrads.

The first limited *rbc*L data (Chase et al., 1993) were more consistent with the first of Vink's (1988) trees in placing *Tasmannia* below *Drimys* and *Belliolum*, but they did not address the position of *Takhtajania*. Analyses of rDNA ITS sequences by Suh et al. (1993) showed unusually low divergence within Winteraceae, indicating either an implausibly recent age for the family or a slowdown in ITS evolution, and the presence of two ITS sequences in *Bubbia*, *Belliolum*, *Zygogynum*, and *Exospermum*, apparently reflecting an unusual persistence of polymorphism in ITS. Although unrooted, the tree obtained was consistent with the basal position of *Tasmannia* and the existence of a *Bubbia-Belliolum-Zygogynum-Exospermum* clade.

The rediscovery of *Takhtajania* has allowed placement of this genus and resolution of the apparent conflicts concerning chromosome number. Karol et al. (2000) included *Takhtajania* in analyses of ITS and *trn*L-F spacer sequences from Winteraceae and Canellaceae. Analyses of *trn*L-F indicated that either *Takhtajania* or *Tasmannia* could be basal in Winteraceae, but *Takhtajania* was basal in trees based on ITS and the two data sets combined. Other relationships within the family were the same as those found by Suh et al. (1993). These results are consistent with Vink's (1988) view that the inflorescences of *Takhtajania* are primitive. However, the finding that the chromosome number of *Takhtajania* may be $n = 18$ (Ehrendorfer & Lambrou, 2000) refutes his suggestion that $n = 13$ in *Tasmannia* is derived from $n = 43$, which does appear to be a synapomorphy of *Drimys* and the remaining groups.

Fossil Record

There are several old reports of fossil winteraceous leaves, but these were not based on a critical analysis of leaf architecture, and they have not

been reexamined recently. As noted above, Winteraceae have irregular "first rank" venation (Hickey, 1977), which has been considered primitive for angiosperms (Hickey & Wolfe, 1975), but otherwise their leaves are not very distinctive. Cuticle analysis might allow better evaluation of these determinations, for example by showing the stomatal plugs characteristic of most genera (Bailey & Nast, 1944; Feild et al., 1998).

Some leaf reports are from areas where Winteraceae occur today and winteroid pollen is known in the fossil record (cf. below). Berry (1938) described *Drimys patagonica* from the early Miocene of Argentina, said to resemble Winteraceae in having a "papillose" lower surface; the venation is consistent with Winteraceae, though probably not diagnostic. Dusén (1908) described *Drimys antarctica* from the Paleocene (Askin, 1992) of Seymour Island on the Antarctic Peninsula; the irregular spacing and angles of the secondary veins are consistent with Winteraceae. Deane (1902), miscited by Praglowski (1979) as Card (1902) and possibly the source of an unreferenced remark by Berry (1938), reported *Drimys* leaves from the Tertiary of New South Wales, but the fragment illustrated has no distinctive features. Leaves of Winteraceae have not been recognized in more recent studies of rich Australian Tertiary floras (Carpenter et al., 1994; Christophel, 1994; McLoughlin & Hill, 1996; R. S. Hill, pers. comm. 1999), which have used more critical methods of leaf identification.

More convincing is a report by Poole and Francis (2000) of vesselless wood described as *Winteroxylon jamesrossi* I. Poole & J. E. Francis from the mid-late Santonian–early Campanian of James Ross Island on the Antarctic Peninsula. Although the exact combination of pitting, ray, and parenchyma features does not occur any modern genus of Winteraceae, all are found within the family, and other vesselless angiosperm taxa (*Amborella*, Trochodendrales) are more different.

A few other megafossil reports are from outside the geographic range of modern Winteraceae and fossil records of winteroid pollen. Chaney and Sanborn (1933) described Oligocene leaves from Oregon as *Drimys americana* R. W. Chaney & Sanborn, but this is questionable, since the venation differs from that of Winteraceae in having thicker, more distinct secondary veins. Page (1979) compared vesselless wood from the Late Cretaceous (Maastrichtian) of California with Old World Winteraceae; she separated it from New World *Drimys* and Trochodendrales based on its abundant parenchyma. Gottwald (1992) described vesselless wood from the Eocene of Germany as *Winteroxylon mundlosi*, which was similar enough to the Antarctic wood of Poole and Francis (2000) that they assigned their material to the same genus. If these fossils are winteraceous, they would imply that the family extended into Laurasia, as in Mexico and Malesia today. However, the possibility that they represent extinct vesselless lines not directly related to Winteraceae should also be considered, given the absence of more diagnostic winteroid pollen, the abundance of Trochodendrales in the Early Tertiary of Laurasia (Crane et al., 1991), and the presence of leaf cuticles with similarities to *Amborella* in the lower Potomac Group (Upchurch, 1984). Extinct vesselless lines should be more common if the lack of vessels in Winteraceae is primitive, since this would imply that the ancestors of many other taxa lower in angiosperm phylogeny were also vesselless.

The record of Winteraceae has been solidified and greatly extended by palynology, based on ulcerate tetrads closely comparable to the family (Figs. 1a, 2). Unlike the wood, the distinctive features of the pollen are clearly derived and thus more indicative of this particular clade. Winteroid tetrads are a persistent but minor element in latest Cretaceous and Tertiary rocks of Australasia (Dettmann & Jarzen, 1990), consistent with the low pollen production and subordinate ecological status of Winteraceae today. Such pollen was tentatively reported (without illustration) by Cranwell (1959) from the Paleocene of Seymour Island, and figured by Couper (1960) from the Oligocene of New Zealand as *Pseudowintera* sp. Couper's material was named *Pseudowinterapollis* by Krutzsch (1970); similar tetrads from the latest Cretaceous through Miocene of southeastern Australia were named *Gephyrapollenites* (with three species) by Stover and Partridge (1973), who were apparently unaware of Krutzsch's article. Martin (1978) indicated that the closest match for the Australian fossils is *Tasmannia*. Mildenhall and Crosbie (1979) extended the range of *Pseudowinterapollis*, which they considered most similar to *Pseudowintera*, from the latest Cretaceous through the Pleistocene of New Zealand. As noted by Suh et al. (1993), since phylogenetic analyses indicate that *Pseudowintera* is nested within the family, these data imply that crown-group Winteraceae (i.e., the clade consisting of all derivatives of the most recent common ancestor of the living members: Doyle & Donoghue, 1993) originated before the end of the Cretaceous. These grains were accepted as Winteraceae by Muller (1981) in his critical review of pollen evidence for extant angiosperm families. In Australasia, the oldest record is from the mid-Campanian

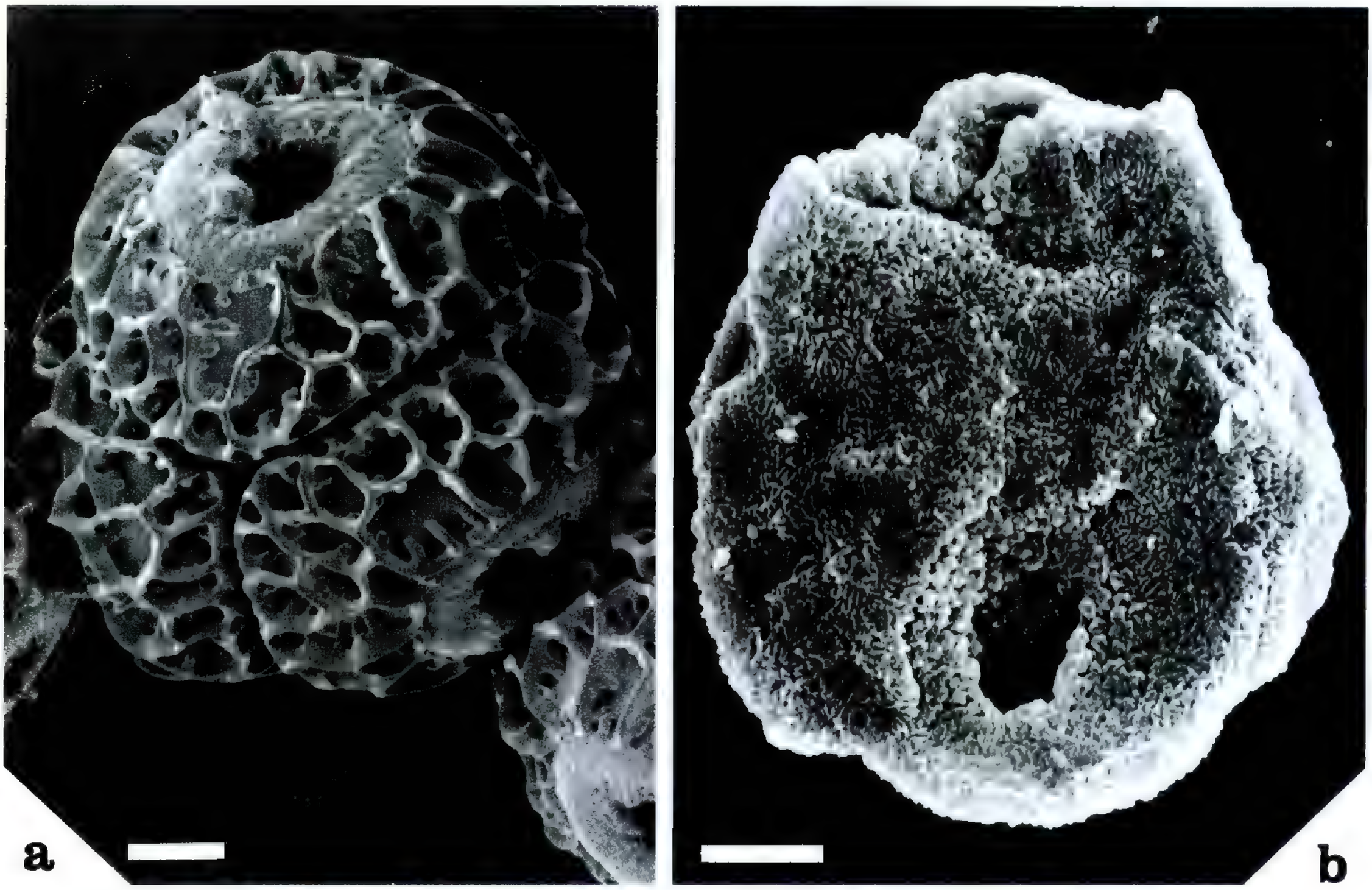

Figure 1. Recent and Early Cretaceous pollen tetrads, SEM. —a. *Drimys winteri* (cultivated, Davis, California). —b. *Walkeripollis gabonensis* (Doyle et al., 1990a), Zone C-VII (late Barremian?), Gabon. Scale bar = 5 μm.

of the Otway Basin of southeastern Australia (Dettmann & Jarzen, 1990). Winteraceae are also known from the Maastrichtian or Paleocene through the Miocene of central Australia (Twidale & Harris, 1977; Harris & Twidale, 1991; Macphail et al., 1994) and the Oligocene of Tasmania (Macphail & Hill, 1994). However, since the early report of Cranwell (1959), Winteraceae do not seem to have been observed in Tertiary pollen floras from Antarctica (Truswell & Drewry, 1984; Truswell, 1991; Askin, 1992).

Mildenhall and Crosbie (1979) also reported loose ulcerate tetrads and monads, named *Harrisipollenites*, from the Oligocene through Pleistocene of New Zealand, which they compared with the monads of *Zygogynum* species (Sampson, 1974). Since phylogenetic analyses indicate that monad-producing *Zygogynum* is one of the most apical branches in the family (Vink, 1988; Suh et al., 1993; Karol et al., 2000), this record is evidence that crown-group Winteraceae had diversified to a high level by the Oligocene. These considerations would further support the view of Suh et al. (1993) that the low divergence of ITS sequences within Winteraceae is due to a slowdown in molecular evolution, rather than a recent origin of the crown-group.

Two types of winteroid tetrads are also known from Tertiary beds (considered early Miocene) of the Cape region in South Africa (Coetzee, 1981; Coetzee & Muller, 1984; Coetzee & Praglowski, 1988), where Winteraceae are now extinct. This implies that *Takhtajania* is the only survivor of a formerly more widely distributed assemblage of Winteraceae in the African-Madagascan region. The same floras contain other taxa that no longer occur in mainland Africa but persist in Madagascar: *Ascarina* (Chloranthaceae), *Casuarina*, Cupanieae (Sapindaceae), and Sarcolaenaceae. However, according to Coetzee and Muller (1984) and Coetzee and Praglowski (1988), these fossils are most similar not to pollen of *Takhtajania*, but rather to that of the Australasian genera *Tasmannia* ("*Drimys*" *piperita*) and *Bubbia* ("*Zygogynum*" *queenslandianum*).

Although pollen of *Drimys* occurs at low frequencies in the Quaternary of Chile (e.g., Heusser, 1981), there have been few reports of winteroid tetrads from older sediments in South America, although such rocks have been extensively studied (cf. Askin & Baldoni, 1998). Apparently the oldest are grains identified by Baldoni (1987) as *Gephyrapollenites calathus* Partridge from the Paleocene-Eocene of Argentina. Barreda (1997) reported *Pseudowinterapollis couperi* Krutzsch from the Oligo-Miocene of Argentina; she provided SEM figures

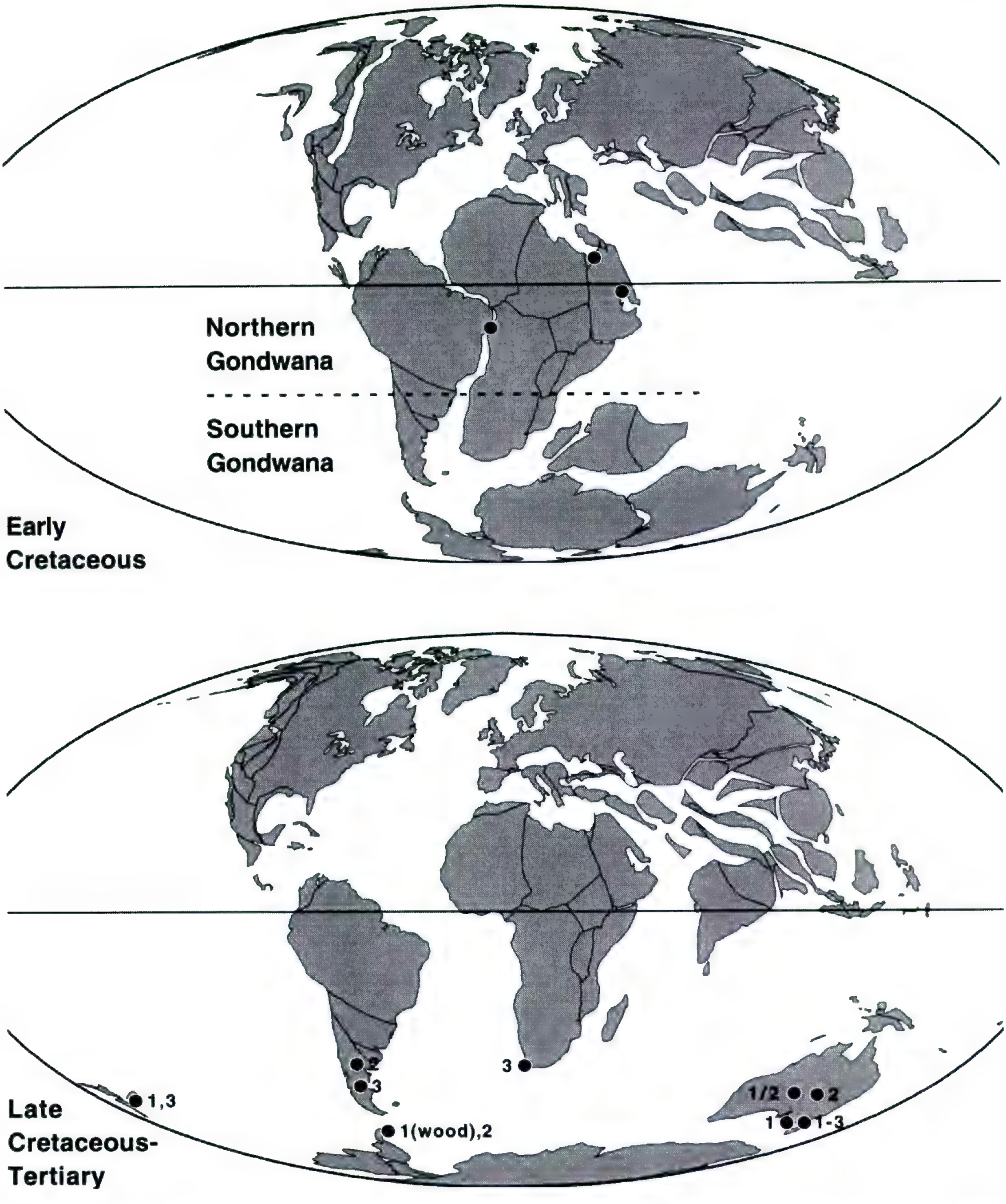

Figure 2. Pre-Quaternary occurrences of ulcerate tetrad pollen related to Winteraceae and vesselless wood of Poole and Francis (2000). Top, Early Cretaceous (base map 120 My, Barremian-Aptian: Scotese, 1997). Bottom, Late Cretaceous and Tertiary (base map 50 My, Eocene: Scotese, 1997); 1: Campanian, Maastrichtian; 2: Paleocene, Eocene; 3: Oligocene, Miocene, Pliocene. See text for references.

showing a tetrad closely comparable to *Drimys* in sculpture and presence of a well-defined annulus.

The stratigraphic and geographic range of winteroid pollen was extended dramatically by Walker et al. (1983), based on tetrads in two cores from the late Aptian-early Albian of Israel. At this time (Fig. 2), Israel was part of the Northern Gondwana province of Brenner (1976), which straddled the Early Cretaceous equator and included all but the southern portions of Africa and South America. In contrast, Late Cretaceous, Tertiary, and Recent occurrences of Winteraceae (except those in Central America and Malesia) are in regions that belonged to Brenner's Southern Gondwana province in the

Early Cretaceous. The Israeli tetrads, designated *Walkeripollis* sp. A by Doyle et al. (1990a), resemble pollen of modern Winteraceae in having a round ulcus consisting of a central pore surrounded by a thicker annulus, underlain by a safranin-staining ring that Walker et al. (1983) assumed was thickened endexine, as in many modern Winteraceae (Praglowski, 1979). However, Walker et al. interpreted them as more primitive than living Winteraceae because of their finer, foveolate-reticulate sculpture.

Still older winteroid pollen was described by Doyle et al. (1990a, b) from the Cretaceous of Gabon, as *Walkeripollis gabonensis* J. A. Doyle, Hotton & J. V. Ward (Fig. 1b). These tetrads appear to be even more primitive in having a slightly elliptical aperture (presumably transitional from a sulcus) and foveolate sculpture, with small tectal perforations only. Transmission electron microscopy (TEM) confirmed that the endexine is thickened under the annulus. These grains were found in only one sample, from an interval (Zone C-VII) near the end of filling of the nascent South Atlantic rift with continental sediments, but they are fairly common in this sample. Their significance was overlooked by Doyle et al. (1977, 1982), who assumed that they were tetrad-producing variants of *Tucanopollis crisopolensis* (Regali, Uesugui & A. S. Santos) Regali, a common angiosperm in these beds, which also has a round, sculptured aperture. The age of Zone C-VII and its equivalents in Brazil was originally considered early Aptian (Doyle et al., 1977, 1982), but Regali and Viana (1989) and Doyle (1992) argued that it is late Barremian, based in part on reports of two associated new groups, *Afropollis* and tricolpate pollen, in independently dated late Barremian rocks in Morocco (Gübeli et al., 1984) and England (Penny, 1989) (and more recently Israel: P. J. De Haan, unpublished Ph.D. thesis, Botany, Univ. California, Davis, 1997).

Compared with modern Winteraceae, both *Walkeripollis gabonensis* and *W.* sp. A are anomalous in being calymmate (Van Campo & Guinet, 1961), with the tectum partially continuous between adjacent monads of the tetrad. In this respect, they appear more advanced than the acalymmate tetrads of the modern taxa, where the tectum stops at the junction between monads. Since the circular aperture and coarser reticulum of *Walkeripollis* sp. A suggest that this species is phylogenetically closer to modern Winteraceae than *W. gabonensis*, Doyle et al. (1990b) inferred that the calymmate condition arose in the common ancestor of the two species and reversed to acalymmate in modern Winteraceae, along with coarsening of sculpture. The fact that *Walkeripollis* is calymmate may be grounds for caution in relating it to Winteraceae, but *W.* sp. A is almost perfectly intermediate between *W. gabonensis* and modern Winteraceae, and both species are only weakly calymmate, implying that the reversal required would be minor. The fact that both *Walkeripollis* species have a conspicuous annulus, like *Takhtajania, Drimys, Belliolum,* and *Zygogynum* species with monads (Praglowski, 1979), suggests that the lack of a well-differentiated annulus in *Tasmannia, Pseudowintera, Bubbia, Exospermum,* and *Zygogynum* species with tetrads is derived. Absence of an annulus seems loosely correlated with smaller aperture size. *Exospermum* and *Zygogynum* are most like *Walkeripollis* in having fine sculpture, but their position in morphological and molecular phylogenies (Vink, 1988; Suh et al., 1993; Karol et al., 2000) implies that this condition is secondarily derived.

Winteroid tetrads may be more widespread in the Early Cretaceous than reported so far. In the middle Albian Khafji member of the Wasia Formation of offshore Saudi Arabia, S. L. Gaponoff (pers. comm. 1990 & 1999) found a single tetrad, which is similar to *Walkeripollis* sp. A in sculpture but looser and apparently acalymmate, suggesting that it is still more closely related to crown-group Winteraceae. But so far there is a gap in the record of such pollen through the first half of the Late Cretaceous.

A possibly related pollen group consists of foveolate-reticulate, ulcerate monads from the early Aptian to Albian of Israel, described by Brenner and Bickoff (1992) as *Retimonoporites operculatus* G. J. Brenner & Bickoff. Their sculpture is roughly similar to that of *Walkeripollis* sp. A; the ulcus is covered by an operculum and underlain by a dark-staining area interpreted as endexine. Because these monads predate *W.* sp. A in Israel, Brenner (1996) interpreted them as representing a more primitive, pre-tetrad state; however, they are probably younger than *W. gabonensis*. It is possible that these grains are related to Winteraceae, but there are enough differences to make this view somewhat speculative. They are much smaller than the monads of *Walkeripollis*, the ulcus is proportionally smaller, and the endexine forms a solid patch rather than a ring around the border of the ulcus.

Doyle et al. (1990a, b) also suggested that two other Cretaceous pollen groups may be related to Winteraceae: *Afropollis*, which is abundant from the late Barremian through the early Cenomanian of Northern Gondwana, and *Schrankipollis*, from the Aptian of Egypt and Maryland. *Afropollis* varies from operculate or zonasulculate in the oldest species to inaperturate in younger ones, with a loose

reticulum surrounding a central body, and the operculates have typical angiospermous columellae below the reticulum. *Schrankipollis* is zonasulculate but elliptical and more finely sculptured. The comparison with Winteraceae was based primarily on the fact that *Afropollis*, *Schrankipollis*, and *Walkeripollis gabonensis* all have finely segmented muri and tend toward circular shape and a round aperture, with the zonasulculus presumably derived by broadening of an operculum. Based on a cladistic analysis of these fossils and pollen of living Winteraceae and Illiciales, Doyle et al. (1990b) inferred that *Afropollis* and *Schrankipollis* belong to an extinct sister group of *Walkeripollis*, Winteraceae, and Illiciales. The two *Walkeripollis* species were successive branches of the stem-lineage leading to both Winteraceae and Illiciales, based on the elliptical aperture of *W. gabonensis* and the finer sculpture of both species. The trichotomosulcate ("syntricolpate") monads of Illiciales were linked with modern winteraceous tetrads by their coarse sculpture, implying that they are secondarily derived from tetrads. Because this analysis placed *Walkeripollis* on the stem-lineage to Winteraceae and Illiciales, and Illiciales have vessels, Doyle et al. (1990b) speculated that the plants producing *Walkeripollis* still had vessels.

This scheme can no longer be defended, since only the tetrads seem securely related to Winteraceae, and molecular evidence against a relationship of Illiciales and Winteraceae has become overwhelming (cf. above). Doyle et al. (1990a, b) acknowledged that the winteraceous affinity of *Afropollis* and *Schrankipollis* was more speculative. They noted that *Afropollis* is anomalous in having a thick endexine all around the grain, as in gymnospermous seed plants, rather than under the aperture only, as in Winteraceae and most other magnoliid angiosperms. Because other characters of *Afropollis* seemed so angiospermous, and because a few magnoliids do have a thick endexine, they suggested that the endexine character may not rule out angiosperm affinities. However, Friis et al. (1999) have found *Afropollis* in separate pollen sacs with no evident angiosperm features. A similar combination of gymnospermous endexine and angiospermous columellae and sculpture also occurs in the Late Triassic Crinopolles pollen group, which Cornet (1989) interpreted as angiospermous, but which Doyle and Hotton (1991) and Doyle and Donoghue (1993) suggested is related to the angiosperm crown-group but more primitive—i.e., on the angiosperm stem-lineage. By the same reasoning, *Afropollis* might also be a side-line of the angiosperm stem-lineage that persisted into the Cretaceous. These doubts do not apply to *Schrankipollis*, since it has only a little endexine under the aperture, but without *Afropollis* to link it with Winteraceae, there is little reason to associate it with the family.

Scenarios for origin of the tetrad pollen of Winteraceae also need revision in light of molecular and morphological evidence that Canellaceae are the sister group of the family. Pollen of Canellaceae is small, round, and monosulcate, with occasional trichotomosulcate variants (Wilson, 1964; Walker, 1976; J.-M. Groult, unpublished D.E.A. thesis, Muséum National d'Histoire Naturelle, Paris, 1998). Its exine structure varies from granular with a continuous scabrate tectum (*Capsicodendron*) to columellar and either foveolate (most genera) or reticulate (*Cinnamosma*). Walker (1976) assumed that the granular extreme is primitive, as part of a general trend in angiosperms as a whole. This would imply that columellae arose independently in Canellaceae and Winteraceae. The idea that granular structure is primitive in angiosperms was supported by the analysis of Donoghue and Doyle (1989), which placed Magnoliales at the base of the angiosperms, but in more recent analyses, where Magnoliales are nested within woody magnoliids, their granular structure is a reversal. Canellaceae are rooted differently in trees based on ITS and *trn*L-F (Karol et al., 2000), but the unrooted tree is the same, and *Capsicodendron* is not basal in either tree. This suggests that the common ancestor of Winteraceae and Canellaceae had small, round, monosulcate pollen with columellae and foveolate sculpture, a type common in the Early Cretaceous. Such pollen may go back to the first angiosperms; for example, it occurs in the near-basal genus *Austrobaileya* (Endress & Honegger, 1980). These data also remove the basis for the conjecture that the parent plants of *Walkeripollis* had vessels (Doyle et al., 1990b). Based on pollen morphology, a phylogenetic analysis would place Canellaceae below *Walkeripollis*, so vessels could have been lost at any point on the line leading to crown-group Winteraceae, either before or after *Walkeripollis*.

Geographic History and Ecological Evolution

Fossil data suggest that the stem-lineage leading to Winteraceae, represented by *Walkeripollis*, originated in the tropical zone of Northern Gondwana. This is consistent with the view of Raven and Axelrod (1974) that angiosperms as a whole originated in this region. The Early Cretaceous climate in the classic areas of Gabon and Brazil has been interpreted as hot and dry (Brenner, 1976; Doyle et al.,

1977, 1982), based on the low frequency of spores, abundance of *Classopollis* (the conifer family Cheirolepidiaceae, noted for its xeromorphic vegetative morphology) and ephedroid pollen (related to modern Gnetales, of which *Ephedra* and *Welwitschia* are desert plants), and the presence of thick Aptian salt deposits, which mark the first influx of marine water into the rift. However, there is evidence for wetter conditions in the Middle East and northern South America, which were near the equator rather than about 15 degrees to the south: less common *Classopollis* and ephedroids, abundant fern spores and Araucariaceae, and occasional coals (Doyle et al., 1982; McCabe & Parrish, 1992; Brenner, 1996). Interestingly, *Walkeripollis* occurs in both areas, suggesting that its parent plants could tolerate some range of rainfall. The fact that *W. gabonensis* is from a rift sequence raises the possibility that its parent plants were growing in cooler upland areas flanking the rift. However, models for rift evolution predict that relief would decrease with time, and although the presence of bisaccate podocarpaceous pollen suggests that there were high elevations near the rift in the earliest Cretaceous, such pollen had disappeared by the time *Walkeripollis* appeared in the Barremian (Doyle et al., 1982).

This scenario also fits the present distribution of probable outgroups of Winteraceae. Canellaceae are entirely African, Madagascan, and American, and Walker (1971) and Raven and Axelrod (1974) interpreted them as of Northern Gondwanan origin. Ecologically, they are more tropical than Winteraceae, and they extend into drier environments (Kubitzki, 1993). The second outgroup to Winteraceae, whether Magnoliales or Piperales, is less well established. However, if Magnoliales are the second outgroup, it may be significant that *rbc*L, *atp*B, and the two genes combined indicate that the basal branch in this group is Myristicaceae (Chase et al., 1993; Savolainen et al., 2000), which have a geographic distribution that also suggests a Northern Gondwanan origin (Walker, 1971; Raven & Axelrod, 1974). Myristicaceae are also a lowland tropical group, and although their species diversity is highest in Asia, Walker and Walker (1981) argued that their most primitive members are the Madagascan genera *Mauloutchia* and *Brochoneura*, which have helical rather than whorled stamens (a view confirmed by a morphological cladistic analysis by H. Sauquet, unpublished D.E.A. thesis, Univ. Pierre et Marie Curie, Paris, 1999).

Thus both fossil distributions and phylogenetic results suggest that the temperate, Southern Gondwana distribution of crown-group Winteraceae came about by southward migration and radiation of plants derived from a stem-lineage in the lowland tropics. In the Early Cretaceous, Southern Gondwana was characterized by Podocarpaceae, Araucariaceae, and abundant spore-bearing plants (Brenner, 1976; Herngreen et al., 1982; Dettmann, 1994), suggesting cool, wet conditions like those where Winteraceae grow today. These data may fit the hypothesis discussed above that vessels were lost in cool, wet environments because of their susceptibility to embolisms caused by freezing (Donoghue & Doyle, 1989; Feild et al., 2000).

A major unresolved problem is when exactly Winteraceae moved into the temperate zone, and by what route. After the records of *Walkeripollis* in the Early Cretaceous, the line is not known until the Santonian-Campanian of Antarctica, represented by *Winteroxylon* wood (Poole & Francis, 2000), and the Campanian-Maastrichtian of Australia and New Zealand (Stover & Partridge, 1973; Mildenhall & Crosbie, 1979; Dettmann & Jarzen, 1990), represented by coarsely reticulate tetrads resembling crown-group Winteraceae. These tetrads seem well established in Australasia, and there are no records of them in the tropics (although given the rarity of earlier reports the significance of such negative evidence can be questioned). Migration to Southern Gondwana would pose little problem in the Early Cretaceous, when the continents were still largely connected (Fig. 2). However, by the time the crown-group is first recognized, ocean barriers had become much wider, especially between Northern Gondwana and Australasia. Possible scenarios may be considered in terms of continental positions ca. 6 My before the Campanian (Fig. 3; Scotese, 1997).

One route to Australasia might be through South America and Antarctica, which was attached to Australia until late in the Cretaceous. On paleogeographic grounds, it is more likely than not that the stem-lineage occurred in South America. In the Early Cretaceous, Brazil and Gabon were two sides of the same rift valley, and given the rarity of *Walkeripollis*, the lack of reports from Brazil is probably not significant. However, if the crown-group spread from South America to Australasia in the Late Cretaceous, it would also have to disperse across the South Atlantic in one direction or the other to explain its Tertiary and Recent occurrences in Africa (Coetzee & Muller, 1984) and Madagascar (*Takhtajania*). This scenario implies that near-basal lines of Winteraceae occurred in South America in the Late Cretaceous, which may conflict with the relatively nested position of the American genus *Drimys* in the family and the rarity of reports of pre-Quaternary winteroid pollen in South America

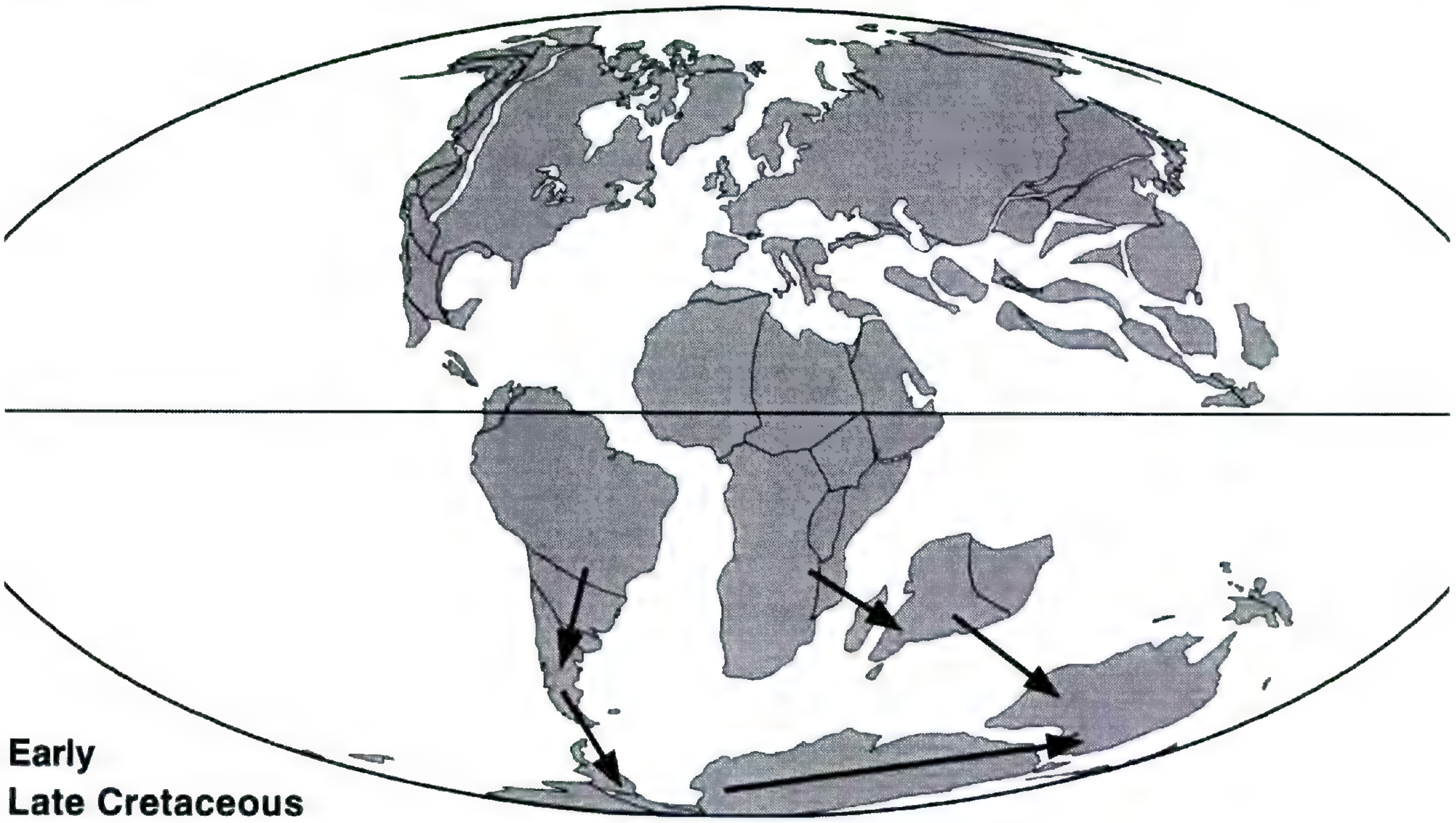

Figure 3. Paleogeography near the time of inferred dispersal of crown-group Winteraceae from Northern Gondwana to Australasia (90 My, Turonian: Scotese, 1997), showing alternative routes through South America-Antarctica and Madagascar-India.

(despite numerous palynological studies: cf. Askin & Baldoni, 1998), as compared with Australasia.

Another route to Australasia might be through Madagascar. Gondwana began to split into East Gondwana (Madagascar, India, Antarctica, Australasia) and West Gondwana (Africa, South America) in the Middle Jurassic (Rabinowitz et al., 1983). However, for some time Madagascar and India, which belonged to the Southern Gondwana province in the Early Cretaceous (Brenner, 1976; Herngreen et al., 1982; Dettmann, 1994), formed a block that might have acted as a stepping stone between Africa and Australasia and/or Antarctica (Fig. 3). This scenario might fit molecular evidence that *Takhtajania* is basal in Winteraceae (Karol et al., 2000): *Takhtajania* would represent a branch that stayed near the original Northern Gondwana area, while the rest of the family was derived from a branch that dispersed to Australasia. *Drimys* could be a line that dispersed later from Australasia to South America via Antarctica (Raven & Axelrod, 1974), consistent with its position in the family.

If, however, Winteraceae in the Tertiary of South Africa were related to modern Australasian genera (Coetzee & Muller, 1984; Coetzee & Praglowski, 1988), more complex scenarios must be envisioned, with a wider Cretaceous distribution of the crown-group and many local extinctions. The discovery of winteraceous wood in the Late Cretaceous of Antarctica (Poole & Francis, 2000) also suggests it would be premature to favor one scenario over another: it implies that Winteraceae reached Antarctica very early, from which they could easily spread to either Australasia or South America. Better evidence on the phylogenetic position of known Cretaceous and Tertiary fossils, or discoveries of new fossils, could greatly clarify the geographic history of the family.

Conclusions

The stem-lineage leading to Winteraceae is one of the oldest recognizable angiosperm lines, extending back to the Barremian stage of the Early Cretaceous in Northern Gondwana. Modern Winteraceae appear to be derived from a line that spread from the tropics into the southern temperate zone, where Winteraceae were widespread in the latest Cretaceous and Tertiary, but the details are uncertain. Major priorities are to fill in the Late Cretaceous record and to clarify the time of appearance of crown-group Winteraceae in Africa-Madagascar, Antarctica, and South America. Hopefully this paper will alert palynologists and paleobotanists working in the Gondwana continents to winteraceous pollen and megafossils, and thus contribute to a more detailed reconstruction of the history of this important family of primitive angiosperms.

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WOOD AND BARK ANATOMY OF *TAKHTAJANIA* (WINTERACEAE); PHYLOGENETIC AND ECOLOGICAL IMPLICATIONS[1]

Sherwin Carlquist[2]

ABSTRACT

Wood and bark anatomy of *Takhtajania* are newly reported on the basis of woody stems to 4.5 cm in diameter, a woody root 4 cm in diameter, and a smaller stem with only a little secondary growth. The wood lacks growth rings and is vesselless. Tracheids bear biseriate or triseriate circular pits or scalariform pits on end walls and sparse circular pits on side walls. Warts and helical thickenings are lacking on the inner surfaces of tracheids. Axial parenchyma is very sparse. Multiseriate and uniseriate rays are about equally abundant, and both are composed of upright cells except for a small number of procumbent cells in multiseriate rays. Ethereal oil cells and sclereids are absent in rays. Bark contains sclereid nests and ethereal oil cells. Wood features closely match those of *Tasmannia* or *Drimys*, in agreement with conclusions reached on the basis of molecular data. Comparative data on bark of Winteraceae other than *Takhtajania* are lacking. Wood features of *Takhtajania* agree with those of subtropical species by lacking features found in temperate Winteraceae (growth rings, warted tracheid surfaces, helical thickenings in tracheids).

Key words: ecological wood anatomy, Magnoliidae, *Takhtajania*, tracheids, vessellessness, Winteraceae.

Wood of Winteraceae has been surveyed at the species level for all genera except *Takhtajania*. The herbarium specimen of *Takhtajania* hitherto available did not have sufficient secondary xylem for satisfactory study; twigs are often of limited value in determining the nature of wood anatomy of a species. Rediscovery of *Takhtajania* has been described in an accompanying paper of this series (Schatz, 2000 this issue).

Winteraceae have traditionally been regarded as retaining numerous character states primitive for dicotyledons (e.g., Dahlgren, 1975; Takhtajan, 1987; Thorne, 1992). Recent molecular studies (e.g., Qiu et al., 1993) place Magnoliales, which contain Winteraceae, at a low level of advancement, but place other orders of flowering plants (notably paleoherbs) as basal to Magnoliales. A primitive status among dicotyledons for paleoherbs has been advanced by Taylor and Hickey (1992). Wood of *Takhtajania* is of potential interest in assessing the position of Winteraceae. The position of *Takhtajania* within Winteraceae can be assessed on the basis of wood anatomy, because wood character states within the family are sufficiently diverse that a key could be constructed on the basis of wood data alone (Carlquist, 1989).

Another phylogenetic question regarding Winteraceae has been raised by the assertion (e.g., Young, 1981) that woody groups of vesselless dicotyledons (Amborellaceae, Tetracentraceae, Trochodendraceae, Winteraceae) may be secondarily vesselless rather than primarily so. The latter view (e.g., Bailey, 1944) has been widely propagated.

Ecological questions also are of significance. The presence of growth rings, warted inner wall surfaces of tracheids, and helical thickenings in secondary xylem tracheids characterize more temperate species of *Drimys* (Carlquist, 1988a), *Pseudowintera* (Patel, 1974; Meylan & Butterfield, 1978), and *Tasmannia* (Carlquist, 1989). These features are lacking in species that occupy subtropical or frost-free sites, as shown by *Belliolum* (Carlquist, 1983a), *Bubbia* (Carlquist, 1983b), *Exospermum* (Carlquist, 1982a), and *Zygogynum* (Carlquist, 1981). The ecology of *Takhtajania*, which grows along a moist ridge in montane tropical Madagascar, has been described in an accompanying paper (Feild et al., 2000 this issue).

Information is offered on bark anatomy of *Takhtajania* here. This information may serve for comparison when studies of bark anatomy, currently lacking (Metcalfe, 1987), are offered.

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Materials and Methods

The first collection of *Takhtajania perrieri* (Capuron) Baranova & J.-F. Leroy made available to me is documented by the collection *Pierre Jules Rakotomalaza et al. 1342* (MO). The collection locality is in the Anjanaharibe-Sud Special Reserve southwest of Andapa in northeastern Madagascar (Schatz, 2000), along a ridge in which many of the *Takhtajania* trees are semi-prostrate and covered with lichens. This collection provided liquid-preserved material of a woody stem 3.5 cm in diameter and a portion of a stem with only a little secondary growth. A second collection from this locality, *Chris Birkinshaw 483* (MO), provided a liquid-preserved stem 4.5 cm in diameter and a root 4 cm in diameter. All of these materials were originally preserved in formalin-acetic alcohol and were transferred to 50% aqueous ethanol.

Sections of the smaller stem and the larger stems and the root (all with both bark and wood) were prepared according to the schedule of Carlquist (1982b). In addition, sections of the stem 3.5 cm in diameter, the stem 4.5 cm in diameter, and the root 4 cm in diameter were prepared on a sliding microtome. Some of these sections were dried between glass slides, sputter-coated, and examined with scanning electron microscopy (SEM). Other sliding microtome sections were stained with a safranin-fast green combination and mounted in Canada balsam. Macerations of the wood of the larger stems and of the root were prepared with Jeffrey's Fluid and stained with safranin. Mean tracheid diameter is based upon tangential diameter of tracheids. Thickness of radial walls is used for measurements of wall thickness. Means are based on 25 measurements except for tracheid wall thickness, in which wall portions judged to be typical were used.

Anatomical Results

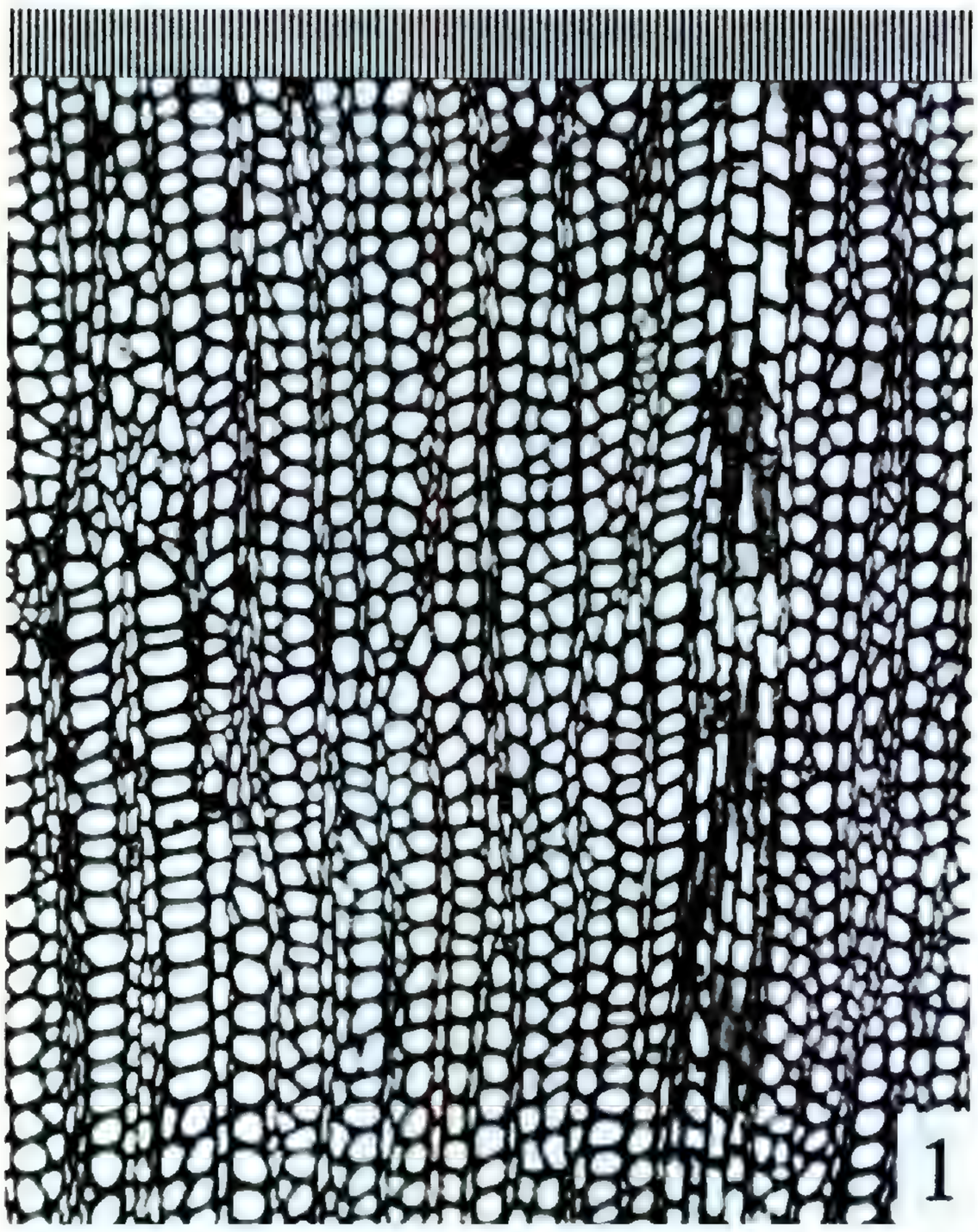
WOOD (FIGS. 1–9)

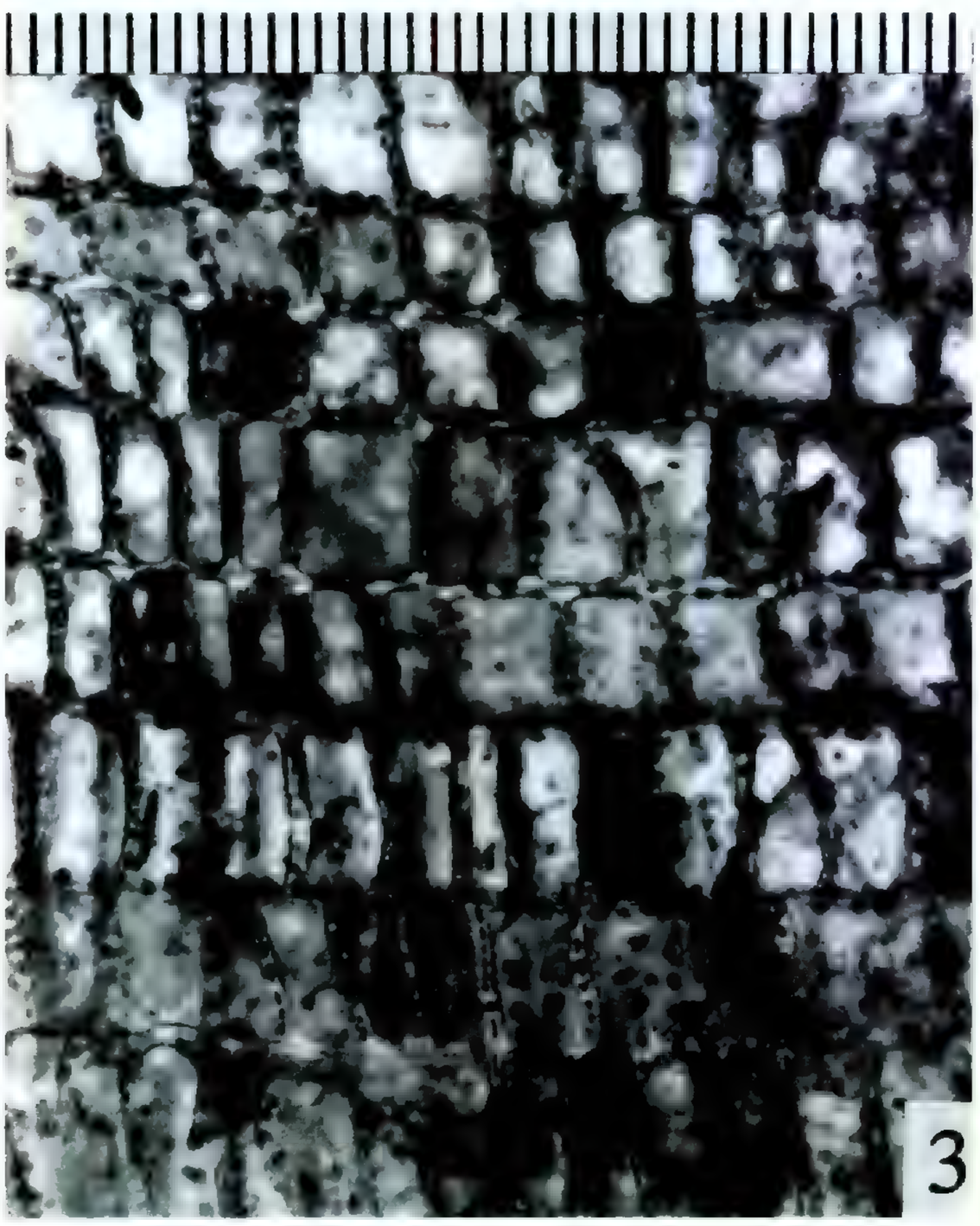
Data are based on mature wood of the stem 3.5 cm in diameter unless otherwise indicated. Quantitative data for the two large stems were not considered significantly different from those of the large root (except for tracheid length), so only data on the stem 3.5 cm in diameter form the basis for the description below.

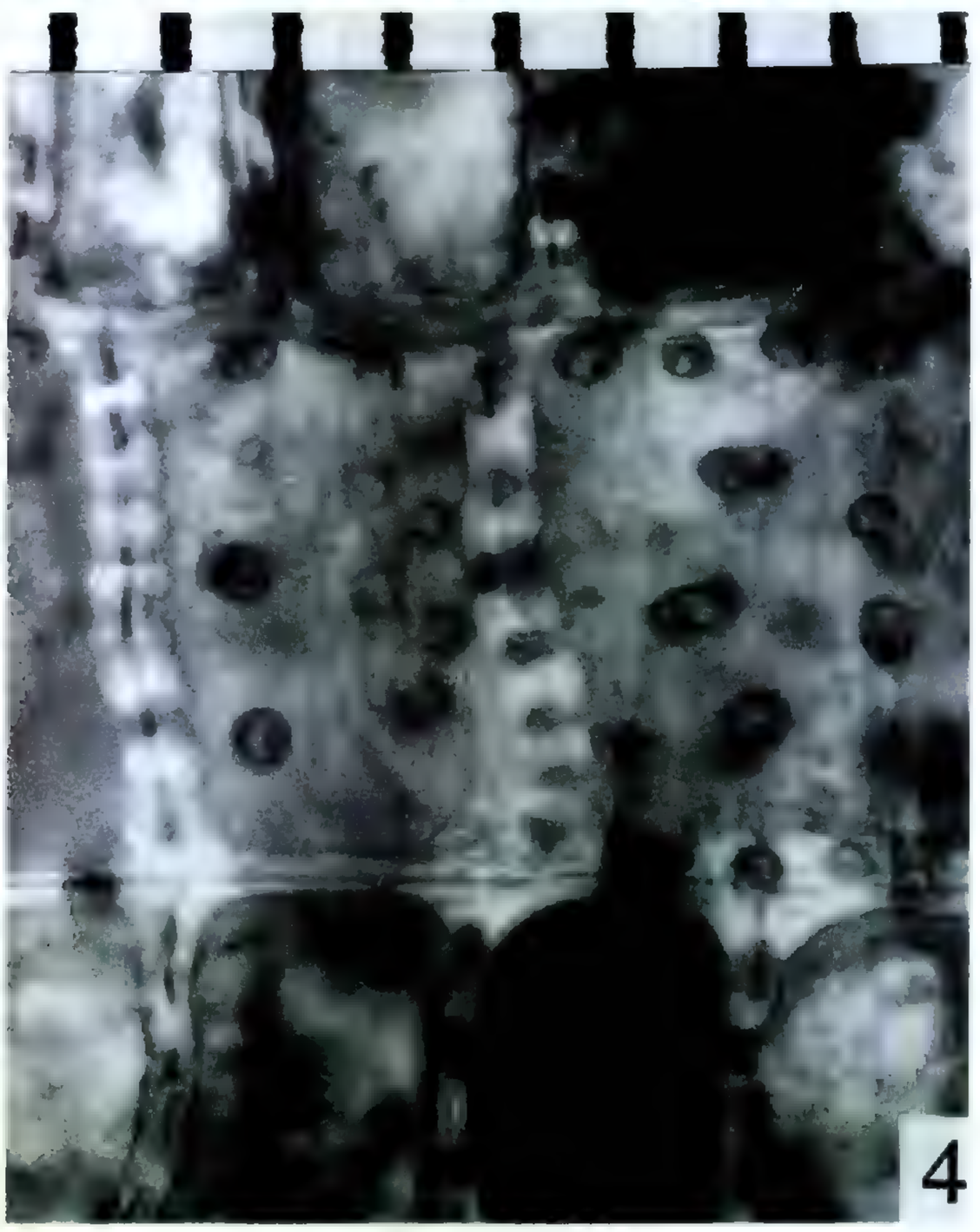
Growth rings absent (Fig. 1). Tracheary elements all tracheids (Figs. 1, 2, 5–9). Mean tracheid length (largest stem), 3002 μm. Mean tracheid length (root), 3821 μm. Mean tracheid lumen diameter, 38 μm. Mean tracheid wall thickness, 4.3 μm. Lateral walls of tracheids with scattered circular bordered pits (Figs. 7, 8). End walls of tracheids with biseriate circular bordered pits or scalariform pits (Figs. 5, 6), the latter sometimes more crowded than shown in Figure 5. Pit membranes present in end wall pits (Fig. 6), both in stems and in root. Tracheids with scalariform pits less common than those with circular pits on end walls formed in small groups with no apparent pattern of distribution within the wood. Tracheids with scalariform end-wall pitting somewhat more common in the root studied than in the stems. Circular pits with narrowly elliptical apertures (Figs. 8, 9), pit cavities about 9 μm in diameter as seen in face view. A small number of tracheids with characteristic elongate elliptical thin areas oriented transversely to helically on inner wall surfaces; these are not like helical thickenings reported in *Pseudowintera* and one species of *Tasmannia* (Carlquist, 1989). Warts absent on tracheid wall inner surfaces (Figs. 5, 8). Pitting generally more abundant on radially oriented walls, but end-wall pitting may be seen on radially oriented and diagonally oriented tracheid walls. Axial parenchyma very scarce (Fig. 7, left), distributed in a diffuse fashion and composed of strands of five or six cells. Rays multiseriate and uniseriate (Fig. 2), about equally frequent (if biseriate rays are included among multiseriate rays). Multiseriate rays are more common than the photograph of Figure 2 suggests because there are long uniseriate wings on most multiseriate rays. Multiseriate rays up to five cells in width at widest point (Fig. 2), mean multiseriate ray width 2.5 cells. Mean multiseriate ray height, 4176 μm; mean uniseriate ray height 1473 μm. Uniseriate rays composed of erect cells; multiseriate rays composed mostly of square to erect cells, a few procumbent cells present (Fig. 3), and the ray type of the species thus intermediate between Heterogeneous I and Paedomorphic I (Carlquist, 1988b: 179). Ray cells with thick lignified walls, most pits bordered as seen either in sectional (Fig. 4) or face view. Borders well developed on pits of tangentially oriented ray cell walls. Some ray cells with dense, granular, dark-staining contents interpreted here as tannins (Figs. 3, 4). Ethereal oil cells and sclereids absent in rays. Wood nonstoried.

BARK

At the periphery of the phloem, a sheath of fibers develops (Fig. 10). These can be termed protophloem fibers. Fibers were not observed within the secondary phloem. In bark of the larger specimens, nests of thick-walled brachysclereids (not illustrat-







Figures 1–4. Sections of wood of *Takhtajania perrieri*. —1. Transection, showing vesselless nature and lack of growth rings. —2. Tangential section; both multiseriate and uniseriate rays are present. —3. Radial section; most ray cells are upright; a few square and procumbent cells are near top of photograph. —4. Portion of ray cells from radial section, showing bordered pits in sectional view and dark-staining contents. Figs. 1, 2, magnification scale above Fig. 1 (divisions = 10 μm); Fig. 3, scale above Fig. 3 (divisions = 10 μm); Fig. 4, scale above Fig. 4 (divisions = 10 μm).

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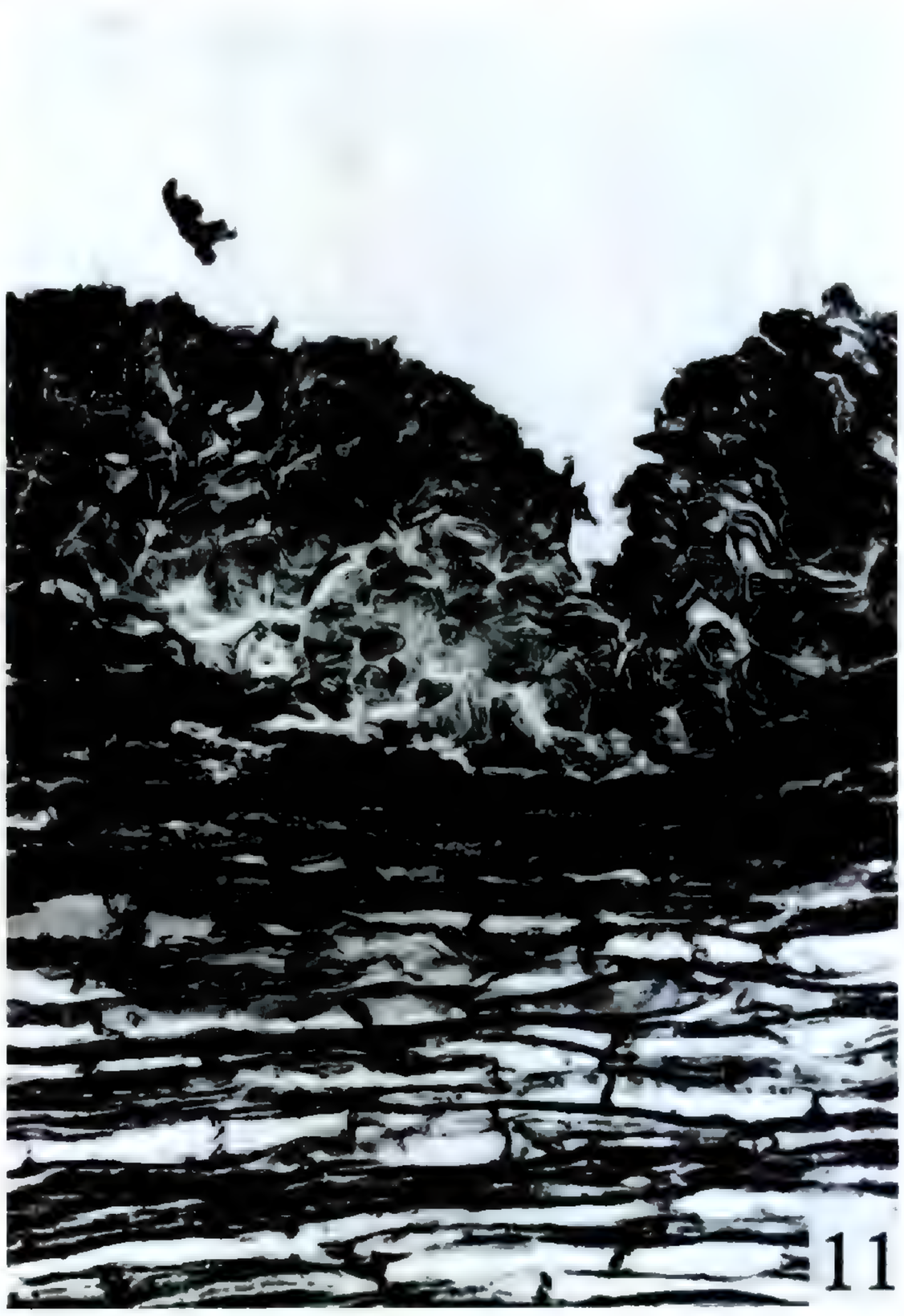
Figures 5–9. SEM photographs (5, 6, 8) and light microscope photographs (7, 9) of wood sections of *Takhtajania perrieri*. —5. End wall of tracheid from radial section, showing both scalariform and circular pits. —6. Scalariform and oval pits from end wall of tracheid of radial section, to show presence of pit membranes, which have been fractured because of handling or preservation method. —7. Radial section, with ray cells at right and a strand of axial parenchyma at left. —8. Inside surface of two tracheids from radial section; walls are smooth. —9. Tracheids from radial section; sparse circular pits in tracheids at left; tracheid to right of center has laterally elongate elliptical thin areas. Figs. 5, 6, 8, magnification scales at upper left (bars = 10 μm). Fig. 7, scale above Fig. 3; Fig. 9, scale above Fig. 4.



Figures 10, 11. Transections of bark of *Takhtajania perrieri*. —10. Section from younger stem, with phloem at left, cortex at right, to show sheath of protophloem fibers (dark). —11. Section from older stem, to show tangentially stretched cortical cells (below) and phellem (above); lamellate walls may be seen in some phellem cells. Figs. 10, 11, magnification scale above Figure 3.

ed here) are present in bark tissue that represents tangentially stretched cortical cells. Scattered ethereal oil cells are also present in this bark tissue.

Phellem is notably thick on the older stems studied (Fig. 11). As seen at higher magnifications, the walls of the phellem cells are clearly lamellate.

Ecological Conclusions

Presence of growth rings, presence of warts on inner walls of tracheids, presence of helical thickenings in tracheids, and to a certain extent, narrowness of tracheids have been interpreted as related to coldness in habitats of particular species of Winteraceae (Carlquist, 1989, and papers cited therein). *Takhtajania* lacks all of these features. From this evidence alone, one could conclude that *Takhtajania* grows in a frost-free zone, in contrast to the habitats of, for example, *Pseudowintera*.

Length of vessel elements is related to ecology in vessel-bearing dicotyledons (Carlquist, 1975). In vesselless angiosperms, as in vesselless gymnosperms, tracheid length is primarily related to diameter of axis from which a wood sample was taken (Carlquist, 1975: 141). The tracheid length recorded here for *Takhtajania* is consonant with that concept, because the sample was taken from a relatively small tree. The greater length of tracheids in the root as compared to those of the stem is in accordance with data by Patel (1965) that root vessel elements are longer than stem vessel elements in dicotyledons at large. Sections and macerations suggested that scalariform end-wall pitting on roots was somewhat more abundant in tracheids of roots as compared to those of stems.

Phylogenetic Conclusions

In the summary of woods of Winteraceae (Carlquist, 1989), a key based on wood of the genera other than *Takhtajania* was presented. Now that *Takhtajania* wood has become available, one can place this genus in the key. *Takhtajania* falls closest to *Tasmannia*. In both genera, scalariform pitting appears in some tracheids (at least in the earlier years of secondary xylem formation), multiseriate rays average between 2.1 and 4.1 cells at widest point, procumbent cells are scarce in mul-

tiseriate rays, and ethereal oil cells are absent in rays. The wood of *Drimys* is similar, differing in having more abundant procumbent cells in rays. These results are interesting in that the molecular data now at hand (Karol et al., 2000 this issue) show greater similarity between *Takhtajania* and *Tasmannia* or *Drimys* than between *Takhtajania* and either *Belliolum, Bubbia, Exospermum, Pseudowintera,* or *Zygogynum.* In view of the fact that molecular results (Karol et al., 2000) show *Takhtajania* to be basal within Winteraceae, wood features of *Takhtajania,* as well as those of *Drimys* and *Tasmannia,* can be expected to exhibit more numerous primitive character states than those of the other genera.

Bark anatomy of *Takhtajania* cannot be compared to bark of other Winteraceae because few data have been accumulated on bark anatomy in the family (Metcalfe, 1987). Esau and Cheadle (1984) have described secondary phloem of Winteraceae in detail, but were not concerned with broader aspects of bark in the family.

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WINTERACEAE EVOLUTION: AN ECOPHYSIOLOGICAL PERSPECTIVE[1]

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ABSTRACT

The Winteraceae have long been regarded as the most primitive living family of angiosperms. The justification for this view partly arises from their lack of xylem vessels. Because xylem vessels have been considered a key innovation in allowing angiosperms to rise to their current ecological dominance, Winteraceae have been portrayed as declining relicts, limited to wet forest habitats where their tracheid-based wood does not impose significant hydraulic constraints. However, phylogenetic analyses indicate that the vesselless wood of Winteraceae may be secondarily derived, while extension of the Winteraceae fossil record into the Early Cretaceous suggests that this family has a more complex ecological history than can be deduced from their current distribution. These observations motivate a re-examination of the functional significance and selective pressures underlying the pattern and direction of character change of the distinctive features of this family. In particular, physiological studies on stomatal plugs and xylem hydraulic parameters are consistent with their ecological success in wet, temperate environments.

Key words: stem water transport, tracheids, *Takhtajania,* Winteraceae, xylem evolution.

The first angiosperms have been traditionally portrayed as slow-growing trees characterized by "primitive" morphological features and confined to wet tropical environments (Bailey, 1944; Takhtajan, 1969; Carlquist, 1975; Cronquist, 1981). According to this woody-tree hypothesis, Winteraceae are the least modified descendents of this hypothetical angiosperm ancestor (van Tieghem, 1900; Thompson & Bailey, 1916; Bailey & Thompson, 1918; Bailey, 1944; Smith, 1945; Takhtajan, 1969; Thorne, 1974). A major reason for this association is the absence of xylem vessels in Winteraceae (van Tieghem, 1900; Bailey & Thompson, 1918; Bailey, 1944; Cronquist, 1981; Carlquist, 1983, 1987, 1996; Gifford & Foster, 1989), although the presence of other putatively primitive morphological characters has been used to reinforce this view (Bailey & Swamy, 1951; Thorne, 1974; Hickey & Wolfe, 1975). A logical extension of this perspective is to view Winteraceae as an evolutionary dead end, confined to wet forest refugia where primitive aspects of their morphology and ecology would not be disadvantageous (Bailey, 1944, 1953; Bailey & Nast, 1945; Smith, 1945; Takhtajan, 1969; Carlquist, 1975, 1983). In particular, Winteraceae have been described as limited to understory habits well suited to their presumably inefficient xylem, with their survival further enabled by the evolution of a distinctive leaf-level feature, i.e., waxy plugged stomata (Bailey, 1944, 1953; Bailey & Nast, 1944; Baranova, 1972; Carlquist, 1975, 1996; Cronquist, 1981).

Recent findings on the phylogeny of angiosperms and the paleoecology and physiological ecology of Winteraceae challenge the idea that Winteraceae represent an unchanged lineage (Young, 1981; Donoghue & Doyle, 1989; Doyle et al., 1990; Chase et al., 1993; Crane et al., 1995; Soltis et al., 1997; Nandi et al., 1998; Feild et al., 1998; Doyle, 1998, 2000; Mathews & Donoghue, 1999). Our goal in this paper is to review Winteraceae's ecological characteristics in relationship to historical changes in their distribution and climatic associations. In particular, we focus on how physiological studies, combined with paleoecological and biogeographic information, alter our perspective on two of the most distinctive features of Winteraceae: stomatal plugs and vesselless wood. The impact of the rediscovery of *Takhtajania perrieri* (Capuron) Baranova & J.-F. Leroy (Schatz et al., 1998) on our understanding of the ecological evolution of the Winteraceae will also be considered.

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Table 1. Classification and geographic distribution of modern Winteraceae. Nomenclature and distributions follow Smith (1942, 1943a, b). Ecological distributions were compiled from Feild (unpublished obs. in Australia, Costa Rica, New Caledonia, and New Zealand), G. Schatz (pers. comm. 1998), Smith (1942, 1943a, b), and Vink (1970, 1977, 1985).

Genus	Distribution	Ecology
Belliolum (6–7 species)	New Caledonia, Solomon Islands	Understory and subcanopy treelets to trees in subtropical lowland rainforest
Bubbia (?33 species)	Australia, Lord Howe Island, New Caledonia, New Guinea, Solomon Islands	Shrubs, treelets, to large trees (15 m+) in tropical premontane, montane cloud forest; in New Caledonia some species on serpentine soils
Drimys (6 species)	Central America, Mexico, South America	Subcanopy and canopy shrubs to trees in tropical montane cloud forest, maritime temperate rainforest, páramos
Exospermum (2 species)	New Caledonia	Large trees in subtropical lowland rainforest
Pseudowintera (2 species)	New Zealand	Subcanopy trees, alpine shrubs
Takhtajania (1 species)	Madagascar	Treelets to subcanopy trees in montane cloud forest (~1000 m)
Tasmannia (5–30+ species)	Australia, Borneo, Indonesia, Philippines, New Guinea	Diverse growth forms in alpine and lowland temperate rainforests, tropical lowland forest, tropical montane cloud forest
Zygogynum (6 species)	New Caledonia	Subcanopy trees in subtropical lowland rainforest and montane cloud forest

Ecological Characteristics of Living Winteraceae

Winteraceae, with 65 species in four to six genera (Smith, 1942, 1943a, b; Vink, 1988; Suh et al., 1993), are a diverse evergreen assemblage with growth forms that include minute-leaved epiphytes, terrestrial alpine shrubs and scramblers, large-leaved understory treelets, and medium-sized (~ 20 m tall) trees (Smith, 1942). Although generally limited to wet habitats (rainfall > 1200 mm/year), they can be found in a variety of habitats ranging from subtropical montane mossy rainforests to cool coastal and montane temperate rainforests, and subalpine and alpine shrubberies. *Tasmannia lanceolata* (Poir.) A. C. Smith is an exception that occurs as a small tree in moist sites such as gullies and stream margins in dry sclerophyll-dominated woodlands (rainfall about 800 mm/year) in Tasmania. The current distribution of Winteraceae follows a southern Gondwanan geographic pattern with most species and genera in Australasia (Table 1; Raven & Axelrod, 1974; Schuster, 1976; Raven, 1980), excepting *Drimys granadensis* L. and *Tasmannia piperita* Hook., which currently extend into regions outside of Gondwana. *Drimys* is the sole genus represented in the New World, where it occurs from cold, wet Magellanic rainforests in southern Chile and Argentina to the tropical and temperate highlands of Mexico (Veblen et al., 1995). Finally, the monospecific genus *Takhtajania* is found only in cool montane forests of northeastern Madagascar (Schatz et al., 1998).

Winteraceae are most diverse in subtropical regions (Vink, 1970, 1977, 1985, 1993), but ecologically more successful in terms of their abundance in temperate environments. Within the subtropics, Winteraceae are found in areas with abundant precipitation and/or low evaporative demand such as along rivers, in understory habitats, and in everwet mossy montane forests (Smith, 1942, 1943b; Vink, 1970, 1977, 1985, 1993; Jaffré, 1995). Populations of many Winteraceae such as most *Bubbia* and *Zygogynum* species in the subtropics consist of only a few individuals (Smith, 1942; Vink, 1977; Pellmyr et al., 1990). A potential contributing factor to this low density may be the tendency for limited seed production due, in part, to infrequent pollinator visits (*Sabatinca* moths, *Palontus* weevils; Pellmyr et al., 1990). Most species of subtropical Winteraceae are tolerant to shade and occur as understory treelets (1–4 m tall) and infrequently as subcanopy trees (to 10 m tall) that become established late during succession (Vink, 1993; T. S. Feild, unpublished obs.). Many of these treelets have exceptionally large leaves (15–30 cm in length is not uncommon especially in *Belliolum*) compared to other Winteraceae from cooler climates, which cause plants to appear "top heavy" and susceptible to breakage (Vink, 1970, 1993; T. S. Feild, unpublished obs.). Another characteristic

of some Winteraceae is their ability to reproduce vegetatively by stem sprouting. *Tasmannia piperita* in New Guinea is reported to reproduce primarily by stem sprouts, while other subspecies are known to reproduce by subterranean stolons (Smith, 1943b; Vink, 1970). Profuse stem sprouting, resulting in multiple-branched plants, occurs in *Bubbia, Drimys, Pseudowintera, Takhtajania,* and most species of *Tasmannia* (Vink, 1970; Raleigh et al., 1994; T. Feild, unpublished obs.; G. Schatz, pers. comm. 1998).

The view of Winteraceae as an ecologically restricted group is difficult to reconcile with its high abundance in wet, temperate rainforest habitats (ca. 3000 mm a year, with frequent frost and some snow). In temperate areas, Winteraceae species can dominate the understory and subcanopy as well as grow in exposed habitats. For example, *Drimys winteri* forms dense subcanopy thickets in coastal Chilean temperate rainforests and occurs frequently as a large canopy tree, 19 m tall and up to 65 cm trunk diameter (Lusk, 1993). Winteraceae are also abundant as individuals in subalpine and alpine communities of Australia, Chile, New Guinea, and New Zealand, where freezing can occur at any time of the year (Vink, 1970; Barry, 1980; Kirkpatrick, 1983, 1997; Veblen et al., 1995). All four major clades within Winteraceae (*Bubbia, Drimys, Pseudowintera,* and *Tasmannia,* as inferred from molecular phylogenetic analyses using ITS rDNA; Suh et al., 1993) contain species that grow as small-leaved (< 1–3 cm long) shrubs and small trees in high-altitude wet montane communities (Smith, 1943a; Hope, 1980; Nunez et al., 1996). In Tasmania, *Tasmannia lanceolata* occurs as an abundant prostrate shrub (e.g., approx. 45% cover) on rocky scree slopes well above the eucalypt and *Nothofagus* tree lines, where it is found alongside conifers such as *Diselma* (Cupressaceae) and *Microstrobus* (Podocarpaceae) (Gibson et al., 1995; Kirkpatrick & Bridle, 1999). *Drimys granadensis* grows as a multiple-branched shrub in *Espeletia*-(Asteraceae) dominated wet páramos of Colombia (Smith, 1943a). In New Zealand, *Pseudowintera colorata* and *P. traversii* grow in the company of conifers on alpine plateaus and mountain tops, where they are gregarious colonizers of landslides (Stewart & Harrison, 1987).

Within these temperate habitats, Winteraceae appear to be good competitors. Rebertus and Veblen (1993) reported that *Drimys winteri* produced a substantial rain of viable seeds, and seedlings grew rapidly in response to forest gap formation, impeding the recruitment and growth of *Nothofagus* (Nothofagaceae) and other vessel-bearing angiosperms. *Drimys* apparently requires large-scale disturbances, such as large gaps produced by wind damage and multiple treefalls, to become established (Rebertus & Veblen, 1993). However, with distance from the coast, *Drimys* abundance decreases rapidly, perhaps reflecting a reduction in water availability (Rebertus & Veblen, 1993). Early succession recruitment of Winteraceae occurs in other temperate taxa. *Tasmannia lanceolata* is an abundant colonizer of open fields on the central basalt plateau of Tasmania, which has frequent frost in the winter (Read & Hill, 1983). This species forms dense thickets in grasslands, understories of dry sclerophyll woodlands (~ 800 mm rainfall a year), and on slopes of wet eucalypt gullies (Vink, 1970; Raleigh et al., 1994). *Tasmannia piperita* dominates forest edges in subalpine rainforests and penetrates into frost-stricken grasslands of high altitudes (> 3000 m) in New Guinea (Vink, 1970; Hope, 1980). *Tasmannia piperita* is also common on landslide-exposed soils in montane habitats (Hope, 1980).

Paleoecological and Paleogeographic History of Winteraceae

To understand Winteraceae's ecological success (in terms of abundance of individual plants) in wet temperate versus subtropical environments, historical patterns of Winteraceae distribution, continental movements, and climatic changes must be considered. The extensive recovery of Winteraceae fossil pollen (tetrads) indicates that Tertiary and Late Cretaceous Winteraceae shared a similar Southern Hemisphere temperate distribution with modern Winteraceae (Coetzee & Muller, 1984; Coetzee & Praglowski, 1988; Dettmann & Jarzen, 1990; Doyle et al., 1990; Dettmann, 1994; Macphail et al., 1994; Doyle, 2000). The oldest Australasian pollen (collected in southeastern Australia) is from the Campanian (Dettmann & Jarzen, 1990), indicating that, at 80 Ma BP, the geographic range of Winteraceae corresponded to the Southern Gondwana flora province of Brenner (1976). This region was a moist, cool temperate region dominated by conifers (Podocarpaceae, Araucariaceae) and later included the austral angiosperms *Nothofagus* and Proteaceae (Axelrod, 1984; Dettmann & Jarzen, 1991; Spicer et al., 1993; Srivastava, 1994; Hill & Scriven, 1995). Specht et al. (1992) suggested that during the Late Cretaceous, Winteraceae, along with Trimeniaceae, *Ilex* (Aquifoliaceae), and Proteaceae, were understory shrubs growing in temperate lowland forests (mean annual temperature ~ 12°C) dominated by podocarps and proteaceous trees (e.g., *Knightia, Macadamia*). These for-

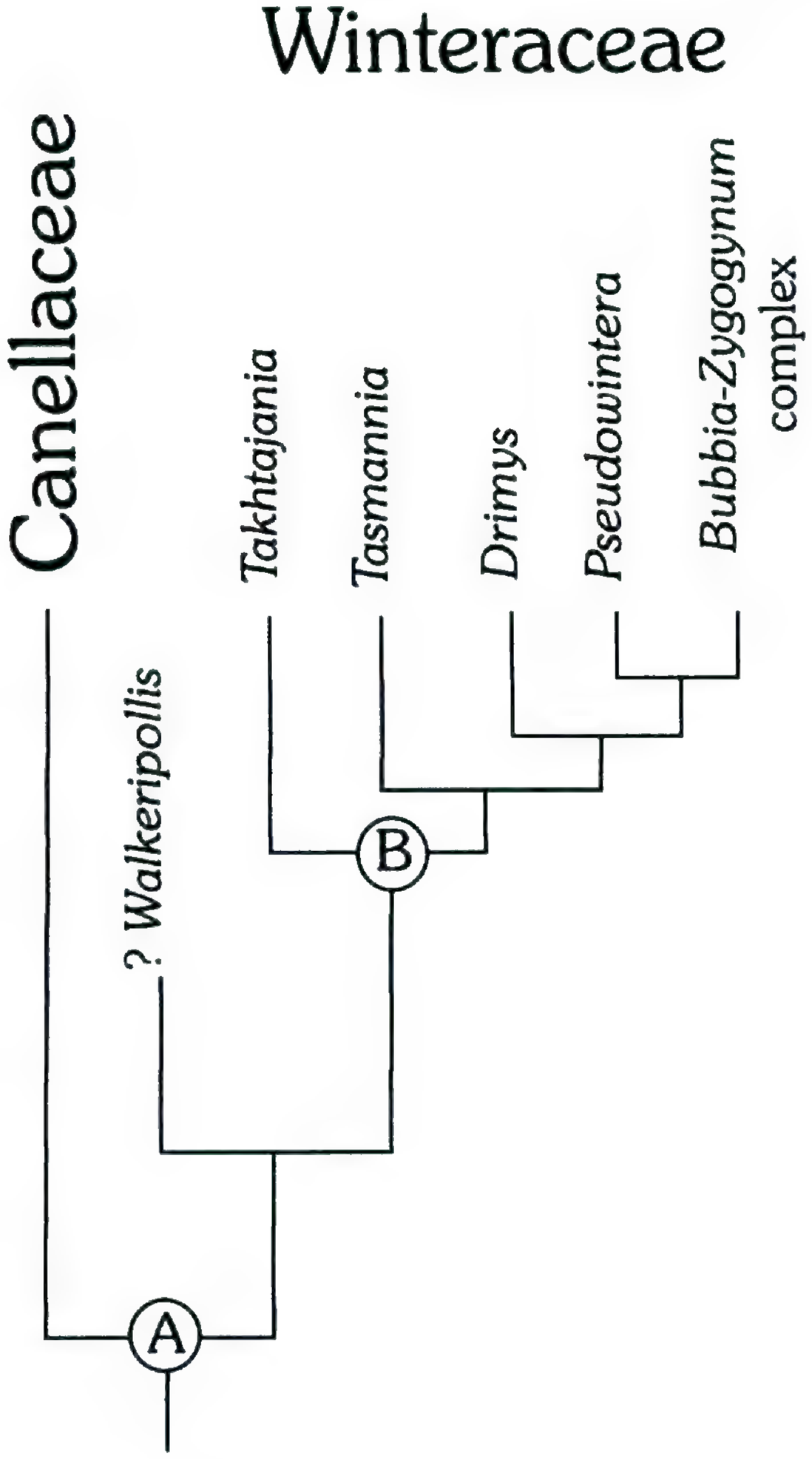

Figure 1. Phylogenetic hypothesis for the relationships among Winteraceae, based on the molecular phylogeny of Suh et al. (1993), the fossil pollen genus *Walkeripollis* (Doyle et al., 1990), and Canellaceae (Chase et al., 1993). Node A is the split from Canellaceae, while node B represents the lineage that gave rise to all extant Winteraceae as well as Late Cretaceous and Tertiary fossil pollen taxa distributed in Australasia (Doyle et al., 1990). *Walkeripollis* may represent an extinct side-branch, found in Northern Gondwana, that pre-dates the origin of node B (Doyle et al., 1990).

est associations occurred in what is now southern Australia and Antarctica; however, no modern analog of this forest community exists today (Specht et al., 1992; Dettmann, 1994; Hill & Scriven, 1995).

Understanding of the climatic and geographic distribution of Winteraceae was greatly enhanced by the discovery of considerably older winteraceous pollen (Late Barremian–Early Albian, 125–105 Ma BP) in several localities well to the north of Southern Gondwana (e.g., Israel and Gabon (Walker et al., 1983; Doyle et al., 1990; Brenner, 1996)). Cladistic studies of these fossils indicate that the plants that produced them were more primitive than the common ancestor of Late Cretaceous, Tertiary, and modern genera of Winteraceae (Fig. 1; Doyle et al., 1990). Surprisingly, these putatively related tetrads occurred within the Cretaceous subarid tropical belt of northern Gondwana (Doyle et al., 1990; Srivastava, 1994; Brenner, 1996; Doyle, 2000). A major implication of these fossils is that the Late Cretaceous Winteraceae and their extant descendents are a temperate branch from an initially tropical lineage that spread southward from Northern Gondwana (Fig. 1; Doyle et al., 1990). Thus, the Australasian regions that include the current center of Winteraceae diversity, such as New Caledonia, (Vink, 1993), are not the cradle of Winteraceae evolution (Dettmann & Jarzen, 1990; Doyle et al., 1990; Dettmann, 1994).

A Northern Gondwanan origin suggests that early Winteraceae were likely to have experienced substantial changes in climate during their migration into the Southern Hemisphere. Two migratory routes into Australasia appear plausible, based on known distributions of fossil winteraceous pollen (Fig. 2; see Doyle, 2000, for more discussion). In the first of these scenarios, Winteraceae reached Australasia through an overland dispersal corridor that included temperate South America and Antarctica (Raven, 1980). An alternative scenario is that the wet temperate Winteraceae lineage originated and radiated in Africa during the Middle Cretaceous (Coetzee & Praglowski, 1988) before dispersing first to Madagascar, and then via overwater dispersal into temperate Australia with Antarctica providing an overland route (Fig. 2B). Presently, it is not clear which of these scenarios is more probable because of the large temporal gap (~ 25 Ma) in fossil pollen data between the first appearance of winteraceous pollen in northern Gondwana and the occurrence of fossils resembling modern Winteraceae in southern Australia (Doyle et al., 1990; Dettmann, 1994; Macphail et al., 1994; Doyle, 2000). Local extinction of Winteraceae, implied by fossil pollen in Africa and central Australia, makes interpretation of their biogeography especially problematic (Coetzee & Muller, 1984; Macphail et al., 1994). Regardless of the route taken to reach Australasia, the fossil pollen record indicates that extant Winteraceae were derived from an originally tropical lineage that migrated into relatively cold areas. This suggests that distinctive features of this family might be associated with success in wet temperate environments.

Stomatal Plugs: Keeping Water In or Out?

Despite the occurrence of most Winteraceae in wet environments, their leaves possess several

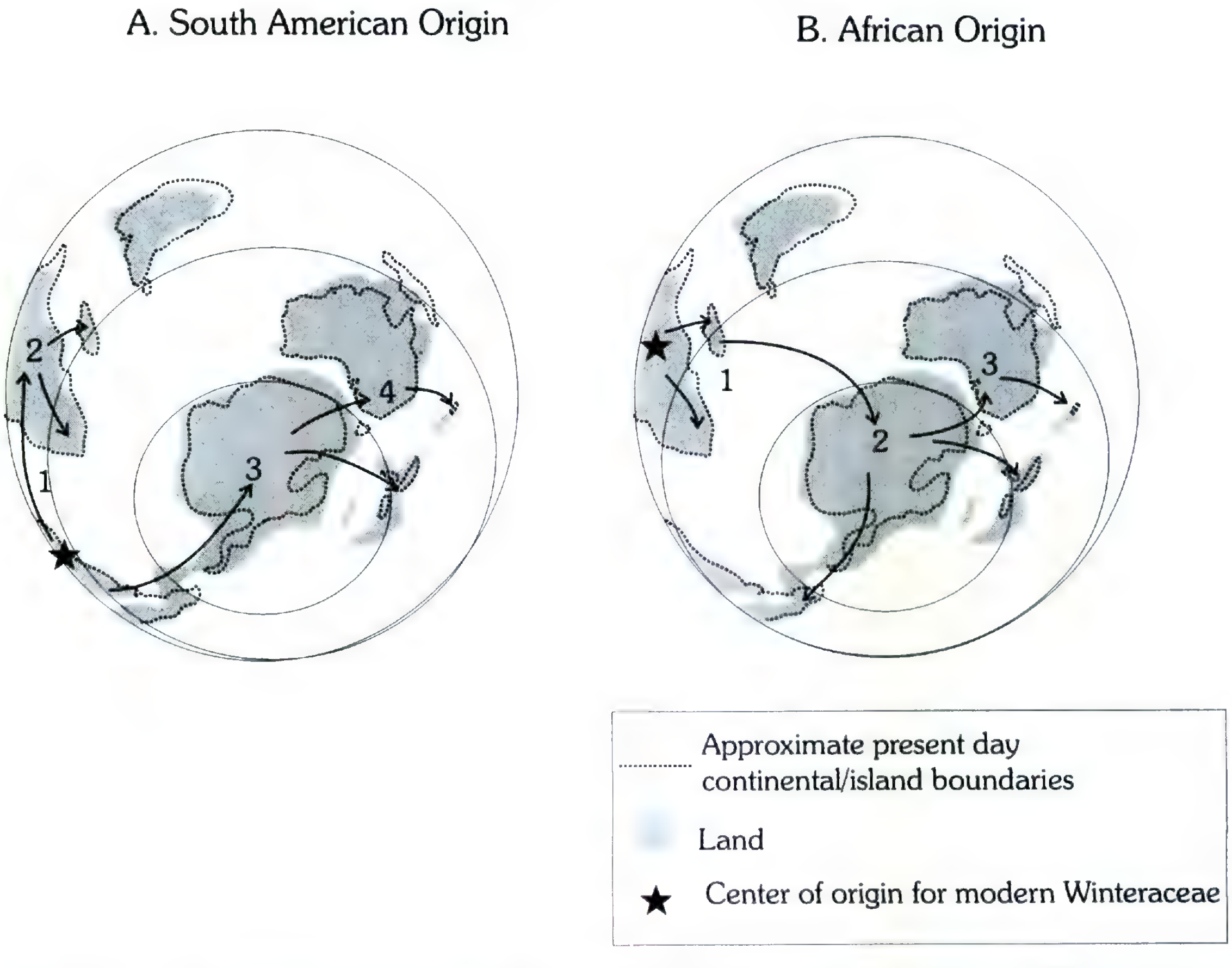

Figure 2. Two paleogeographic hypotheses for the center of origin and routes of migration of Winteraceae into Australasia. The arrangement of continental areas is from the Late Cretaceous (Early Campanian, 80 Ma BP) when fossil pollen closely resembling modern "crown-group" Winteraceae first appeared (Dettmann & Jarzen, 1990). —A. In the first route, Winteraceae originated in temperate regions of southern South America following dispersal from Northern Gondwana (1). To account for Winteraceae pollen in Africa and *Takhtajania* in Madagascar, dispersal to Africa and then Madagascar must be invoked (2). Winteraceae would then have moved across Antarctica into Australasia (3 and 4). —B. An alternative route may have involved the origination of temperate Winteraceae in Africa (possibly southern areas) with *Takhtajania* as a surviving descendant from this radiation. Winteraceae may have then dispersed overwater or possibly overland into Antarctica (1) and finally into other Australasian regions (2 and 3). Stars indicate the centers of origin for node B Winteraceae (see Fig. 1).

seemingly xeromorphic features. To varying degrees, Winteraceae leaves have thick cuticles and/or the stomata are sunken into the abaxial leaf surface (Bailey & Nast, 1944; Baranova, 1972; Bongers, 1973). Perhaps the most striking manifestation of Winteraceae's apparent xeromorphy is the obstruction of each stomatal pore with a granular "plug" composed of cutin and wax (Fig. 3; Bailey & Nast, 1944; Baranova, 1972; Bongers, 1973; Feild et al., 1998). These structures are responsible for the characteristic white reflective appearance of the undersurfaces of Winteraceae leaves (Bailey & Nast, 1944).

The diversity of stomatal ornamentation found in Winteraceae is enormous (Bongers, 1973). In *Takhtajania* and some species of *Tasmannia,* stomatal plugs are absent (Fig. 3A; Baranova, 1972; Bongers, 1973). In others (most *Tasmannia* species), the stomata are only partially occluded by the presence of small crystalline wax rodlets confined to the guard cell rims (Vink, 1970; Bongers, 1973). The abaxial epidermal surfaces of *Drimys brasiliensis* Miers and some entities of *Tasmannia piperita* are covered with dense overarching papillae formed from extensions of the cuticular layer (Smith, 1943a; Bailey & Nast, 1944; Bongers, 1973). Finally, in some *Bubbia* and *Zygogynum* species, the stomatal apparatus is completely buried under a mat of encrusting wax rodlets and an additional layer of cuticle such that the stomata cannot be seen (Bongers, 1973; Metcalfe, 1987; Vink, 1993).

A long-held belief has been that stomatal plugs and the other apparently xeromorphic leaf features of Winteraceae function to restrict water loss (Bai-

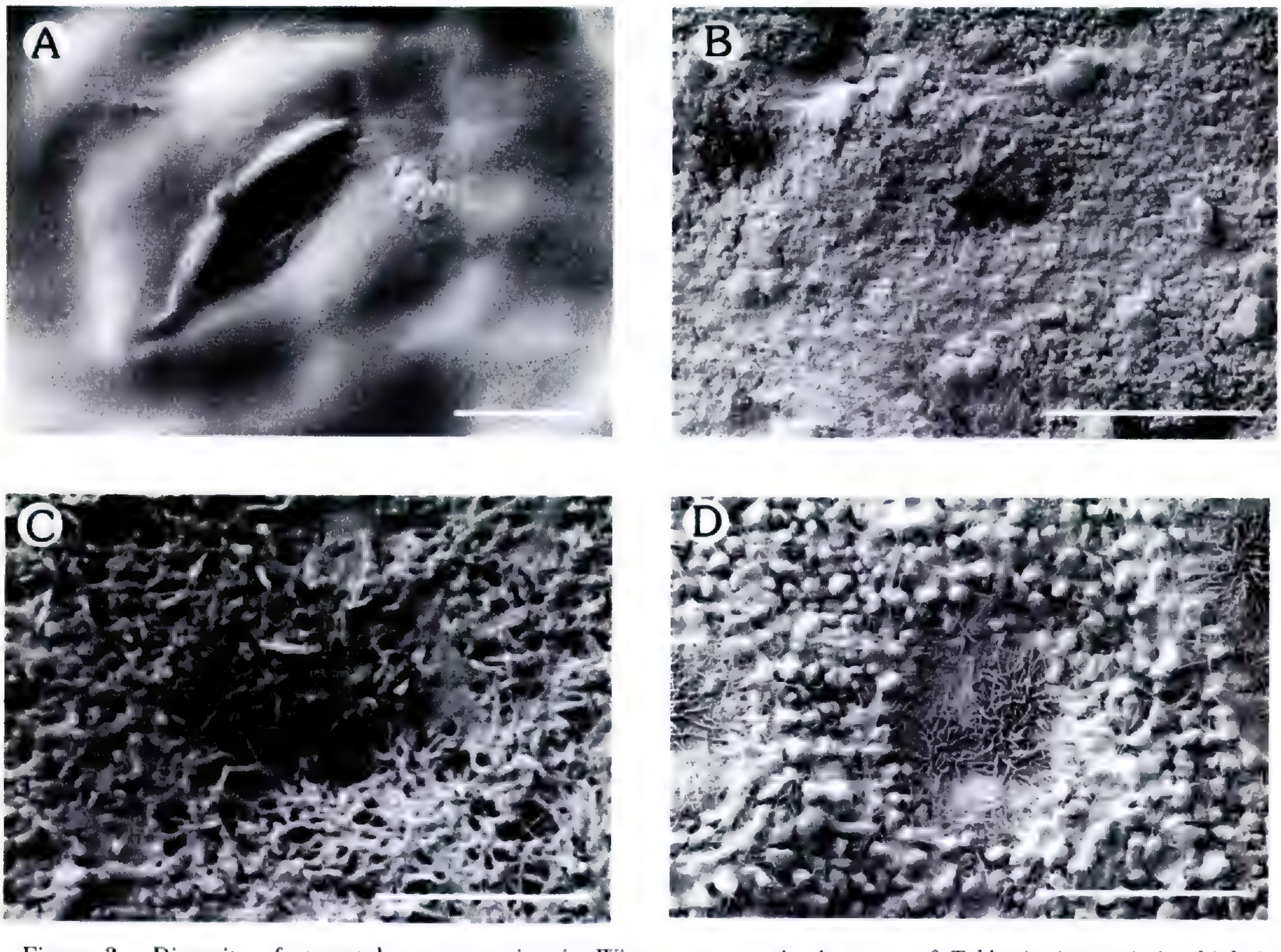

Figure 3. Diversity of stomatal ornamentation in Winteraceae. —A. A stoma of *Takhtajania perrieri*, which is completely free of waxy obstruction. —B. Epidermal surface of *Drimys granadensis*, which is covered with pieces of cuticle and granular waxes. The stomata, which are indicated by the dark clefts, reside below the epidermal layer. —C. Close-up of porous crystalline rodlets associated with a stoma of *Pseudowintera axillaris* (J. R. & G. Forst.) Dandy. —D. *Belliolum rivularis* (Parmentier) Burtt epidermis, which has a dense mat of cuticular papillae (visible between the stomatal regions) and a concentration of tubes (possibly composed of wax or cutin associated specifically with the stomatal apparatus). Scale bars are: 5 μm (A & C) and 20 μm (B & D).

ley, 1944, 1953; Bailey & Nast, 1944; Baranova, 1972; Carlquist, 1975; Cronquist, 1981). As antitranspirants, these structures were proposed to have evolved to compensate for the presumed inferiority of a tracheid-based transport system (see below; Bailey, 1944, 1953; Bailey & Nast, 1944; Baranova, 1972; Carlquist, 1975; Cronquist, 1981; Sperry, 1995). Stomatal plugs also occur in most Southern Hemisphere conifers, such as Araucariaceae, Cupressaceae, Podocarpaceae, as well as many northern groups, which has fueled discussions on a functional linkage between stomatal plugs and the absence of xylem vessels (Jeffree et al., 1972; Yoshie & Sakai, 1985; Wells & Hill, 1989; Stockey et al., 1992; Carlquist, 1996; Stockey & Atkinson, 1993; Stockey & Frevel, 1997; Brodribb & Hill, 1998; Stockey et al., 1998).

The hydraulic compensation argument has been applied to Winteraceae for many years, despite the absence of any information on how stomatal plugs affect rates of leaf gas exchange. In contrast to what their name suggests, stomatal plugs are not solid, but riddled with air-filled pores formed between tubes of cutin and granular wax crystals (Bongers, 1973). Although a porous construction is necessary for CO_2 uptake, this fact seems to have been overlooked in arguments of presumed transpiration-retarding effects. Recently, Feild et al. (1998) examined how these structures influence water loss rates from *Drimys winteri* var. *chilensis* (DC.) A. Gray leaves. Comparisons of plugged and "unplugged" leaves (leaves from which stomatal plugs and associated epicuticular waxes were experimentally removed) demonstrated that under high relative humidity (85%) plugs posed a relatively small resistance to leaf water loss (Feild et al., 1998). Maximum stomatal conductances of *Drimys* leaves with stomatal plugs were about 10% lower than leaves from which the stomatal plugs had been experimentally removed. Maximum conductances of plugged leaves (approx. 100 mmol H_2O m^{-2} s^{-1}), although lower than many lowland rainforest trees

and crop plants, were similar to those reported from other montane cloud forest angiosperms (Feild et al., 1998). With exposure to progressively greater evaporative demand, the water loss rates of *Drimys* leaves with plugs compared to ones without plugs markedly diverged. Unplugged leaves exhibited a 70% decline in stomatal conductance with decreasing ambient relative humidity (from 105 to 35 mmol H_2O m^{-2} s^{-1} over 85% to 40% relative humidity drop). This degree of stomatal closure in response to dry air is similar to that reported for a wide variety of plants lacking stomatal occlusion. In contrast, stomatal conductances of plugged leaves decreased 10% over the same drop in relative humidity, from 100 to 90 mmol H_2O m^{-2} s^{-1} (Feild et al., 1998). The mechanism by which stomatal plugs prevent stomatal closure is unclear. It is possible that plugs may interfere with stomatal movements by maintaining a water vapor concentration around the guard cells that uncouples the guard cells from ambient conditions, or plugs may retard stomatal closure by physically interfering with guard cell movements (Feild et al., 1998). Regardless of the exact mechanism, these results provide physiological evidence that stomatal plugs in *Drimys* cannot be considered adaptations for drought.

In sharp contrast to their traditionally assigned role as anti-transpirants, the functional significance of stomatal plugs appears to be related to the occurrence of Winteraceae in areas that are generally wet (rainforests and cloud forests). One of the inevitable consequences of frequent rainfall and cloud cover is prolonged wetting of leaf surfaces (Brewer & Smith, 1997). Because CO_2 diffuses through water 10,000 times more slowly than in air, water films on leaf surfaces can create a high resistance to CO_2 diffusion into leaves for photosynthetic carbon gain. Photosynthetic measurements of *Drimys* leaves exposed to mist demonstrated that stomatal plugs allow relatively unperturbed photosynthetic activity when leaves are exposed to mist (Feild et al., 1998). Specifically, photosynthetic electron transport rates decreased approximately 40% following misting of *Drimys* leaves that lacked stomatal plugs. In contrast, electron transport rates of plugged leaves were unaffected by misting. Stomatal-associated waxes and cutin allow for continued gas exchange when leaves are wet because water droplets are repelled from the stomatal apparatus (Feild et al., 1998). These water-epidermis interactions keep the stomatal pore and intercellular spaces from being filled with water, which can occur when leaves are exposed to fog (Brewer & Smith, 1997). Further support for the efficacy of stomatal waxes in shedding excess water has been observed in *Tasmannia lanceolata*. Small trees of *T. lanceolata* that occur in eucalypt gallery forest understory and subcanopy, where they encounter frequent leaf wetting from canopy drip, have wax rodlets associated with their guard cells. In contrast, shrubby forms of this species growing on exposed ridge tops in alpine heathlands, which could presumably benefit from transpiration restriction, lack any epicuticular waxes associated with the stomatal apparatus (Feild, unpublished obs.).

These functional considerations provide a new perspective of the selective forces that may have driven the evolution of stomatal occlusion in Winteraceae. The observation that stomatal plugs, at least in *Drimys*, do not apparently protect leaves from drought is not consistent with the idea that these structures are adaptations that have played an important role in the persistence of a "primitive" vesselless phenotype. Further support for the new interpretation of stomatal plugs is that similar structures have evolved in other plants with vessels (such as some Myristicaceae and Epacridaceae; Koster & Baas, 1981), and without vessels (as in many rainforest conifers). All these plants occur in wet forest habitats. In addition, other vesselless angiosperms such as *Amborella*, *Tetracentron*, *Takhtajania*, and many conifers from drier habitats, lack stomatal plugs or other waxy structures obstructing the stomatal apparatus (Fig. 3A; Metcalfe, 1987; Brodribb & Hill, 1998).

Vesselless Wood: Hydraulic Limitation or Enhanced Freezing Tolerance?

Perhaps the best-known feature of Winteraceae is the lack of water-conducting xylem vessels in their wood (Bailey & Thompson, 1918; Cronquist, 1981; Gifford & Foster, 1989). Instead, the water-conducting system of Winteraceae is composed entirely of tracheids. Vessels are also absent in a number of other angiosperm groups such as *Amborella* (1 species), *Tetracentron* (1 species), and *Trochodendron* (1 species). Tracheids are xylem cells that lack cytoplasm such that their lumen provides an unimpeded path for water flow. Water movement between tracheids, however, requires that water must traverse cell walls (Zimmermann, 1983). The primary path for water movement between tracheids are pit depressions in the secondary wall, which although porous in construction, provide some resistance to flow (Zimmermann, 1983). In contrast, xylem vessels are water-conducting tubes generally larger than tracheids both in length and diameter (Carlquist, 1975; Zimmermann, 1983). Vessels consist of files of cells, with

each termed a vessel element, that unlike tracheids have substantially modified axial walls or perforation plates that allow for a more open pathway of water movement (Bailey, 1944; Carlquist, 1975; Zimmermann, 1983; Gifford & Foster, 1989). Vessel elements were apparently derived by a modification of the developmental program giving rise to tracheids, such that the pit membranes were hydrolyzed between the end walls of adjacent cells (Gifford & Foster, 1989). Vesselless wood has traditionally been considered a retained ancestral feature in angiosperms (van Tieghem, 1900; Bailey & Thompson, 1918; Bailey, 1944, 1953; Carlquist, 1975, 1983; Cronquist, 1981). What has propelled this idea is the assumption that vessels represent such an adaptive advantage that their subsequent loss is highly improbable (Carlquist, 1975). With the evolution of xylem vessels, botanists have argued that the early angiosperms were able to exploit habitats with uncertain water supplies and develop growth-enhancing traits such as large, undissected leaves and greater gas exchange rates (Carlquist, 1975; Doyle & Donoghue, 1986; Bond, 1989). By this perspective, hydraulically compromised plants such as Winteraceae have escaped extinction only by being restricted to wet cloud forest habitats (Carlquist, 1975, 1983, 1987).

The view that Winteraceae are primitively vesselless has been challenged by phylogenetic analyses (Young, 1981; Donoghue & Doyle, 1989; Chase et al., 1993; Mathews & Donoghue, 1999). Cladistic analyses indicate that the first angiosperms had vessels (albeit primitive ones), which were subsequently lost in several early angiosperm lines (Young, 1981; Donoghue, 1989; Donoghue & Doyle, 1989; Doyle, 1998). Although molecular phylogenies imply that *Amborella* and Nymphaeales are likely to be basal angiosperm branches and thus primitively vesselless (Mathews & Donoghue, 1999), both Winteraceae and Trochodendrales are nested within clades containing plants with vessels. Winteraceae are most closely related to Canellaceae, and Trochodendrales are included within eudicots (Chase et al., 1993; Soltis et al., 1997). To assume irreversible vessel evolution requires more evolutionary steps by additional independent origins of vessels in angiosperms (Young, 1981; Donoghue & Doyle, 1989). While the parsimony debt incurred by assuming that vessels cannot be lost was not overwhelming, this conclusion implies that the functional arguments for such an assumption should be examined (Donoghue & Doyle, 1989; Mathews & Donoghue, 1999). The cladistic arguments supporting vessel loss in Winteraceae have received much skepticism, primarily on the grounds that no viable mechanism could drive the evolutionary loss of vessels, other than a shift to aquatic environments, as this would entail a shift to an inefficient hydraulic system (Carlquist, 1983, 1996). However, claims about the likelihood of vessel loss in Winteraceae need to be evaluated in a physiological context.

In support of this view, Donoghue and Doyle (1989) argued that little support exists for the view that vessel loss in Winteraceae is likely to have been selected against. First, the forests with low evaporative demand where most Winteraceae currently occur were suggested to be the type of habitats where any decrease in xylem transport capacity by vessel loss would have a relatively small effect (Donoghue, 1989; Donoghue & Doyle, 1989). Donoghue (1989) also suggested that the first vessels, hypothetically present in an ancestor of Winteraceae, were presumably of a primitive morphology containing numerous scalariform partitions, limited in number, and in a background of functional tracheids (Donoghue, 1989; Donoghue & Doyle, 1989). At this stage of low evolutionary specialization, the loss of vessels may not have been difficult, entailing only the retention of pit membranes. For example, the structural distinction and therefore the potential hydraulic consequences of having only tracheids versus having both tracheids and vessels is less obvious when Winteraceae are compared with their closest extant relatives, Canellaceae. Most Canellaceae vessel elements are similar in size and overall shape to tracheids of Winteraceae (Wilson, 1960). Tracheid diameters (16–69 μm; Patel, 1974; Meylan & Butterfield, 1982; Carlquist, 1981, 1982, 1983, 1988, 1989) of Winteraceae overlap with the lower range of vessel element diameters reported from Canellaceae (20–140 μm; Wilson, 1960; Metcalfe, 1987). Xylem conduits in Winteraceae and Canellaceae differ in that the pit membranes are retained in tracheids of Winteraceae, while in vessel elements of Canellaceae scalariform perforation plates are developed. Some Winteraceae species show an intermediate condition where the tracheid pit membranes are partially dissolved, forming relatively large porosities (0.05 μm diam.) as in some species of *Bubbia* (Carlquist, 1983). However, the comparative hydraulic properties of Canellaceae and Winteraceae xylem are not known.

Although it is difficult to determine the importance of specific selective pressures in the past, functional and physiological studies can indicate what factors might have been involved. Combined with information from historical biogeography, functional analyses also provide a context toward inter-

preting phylogenetic inferences about the past ecological and climatic associations of a lineage (Doyle et al., 1990). One common characteristic of the current distribution, ecological abundance, and paleomigratory patterns of Winteraceae is an association with cool, wet temperate environments. Paleoclimatological evidence suggests that at the time Winteraceae moved into Australasia, these southern high-latitude regions may have been colder than previously suspected, perhaps including some regions with persistent snow cover and frozen ground (Rich et al., 1988; Ditchfield et al., 1994; Sellwood et al., 1994; Stoll & Shrag, 1996). Where freezing temperatures are common, tracheids appear advantageous over vessels in terms of their resistance to freezing-induced cavitation (Hammel, 1967; Sucoff, 1969; Sperry & Sullivan, 1992; Sperry et al., 1994; Tyree et al., 1994; Davis et al., 1999). When stems freeze, the insolubility of dissolved gases in ice results in air bubbles. Upon thawing, these bubbles act as nucleation sites for the formation of air-embolisms blocking the movement of water through stems (Hammel, 1967; Sperry & Sullivan, 1992; Hacke & Sauter, 1996; Davis et al., 1999). The probability of a freeze-thaw event resulting in xylem embolism correlates with conduit volume (Sperry & Sullivan, 1992; Sperry, 1995; Davis et al., 1999). This is because a greater conduit volume results in both more numerous and larger air bubbles, which can easily expand to fill the entire xylem conduit during the thaw of xylem sap.

Most conifers are resistant to freeze-thaw-induced cavitation irrespective of the number of freezing events experienced because of their tracheid-based vascular system (Hammel, 1967; Sucoff, 1969; Robson et al., 1988; Sperry & Sullivan, 1992; Sperry et al., 1994). In tracheids, the bubbles produced in frozen sap are sufficiently small that they are collapsed by surface tension during thawing. In contrast, many vesselled angiosperms, and especially those with large conduit volumes, embolize extensively following one or more freeze-thaw events (Sperry, 1995; Pockman & Sperry, 1997; Davis et al., 1999). The tradeoff between vulnerability to freezing-induced cavitation and conduit size may have influenced the direction of xylem evolution in Winteraceae (Donoghue & Doyle, 1989; Donoghue, 1989). Indeed, Winteraceae have been documented to be relatively more frost tolerant compared to co-occurring plants with vessels. Sakai et al. (1981) demonstrated that the freezing resistance of buds, leaves, and stems of *Tasmannia lanceolata* was greater than most co-occurring angiosperms that had vessels (e.g., *Nothofagus, Eucalyptus,* and several Proteaceae) and was similar to that of co-occurring conifers such as *Diselma, Microcachrys,* and *Phyllocladus. Drimys winteri* in southern Chile is also reported to be more tolerant to frost than *Nothofagus* species growing in the same environment (Alberdi et al., 1985). One part of the observed tolerance of Winteraceae to freezing may be the ability of a tracheid-based xylem to avoid freezing-induced limitations on stem water transport.

Despite the central position of xylem structure in discussions on the evolution of Winteraceae, no published reports exist for the hydraulic performance in the field. Clearly, comparative studies of xylem properties and water flux rates under field conditions are needed. However, the selective forces on wood evolution are not confined to hydraulic efficiency. Safety, with respect to avoidance of air embolisms, and mechanical strength are two additional selective pressures on the direction and pattern of xylem evolution in plants (Tyree et al., 1994). Certainly smaller xylem conduits offer higher resistance to water flow. Nonetheless, in colder climates tracheids appear to increase the overall hydraulic capacity by minimizing embolisms by freeze-thaw events (Sperry et al., 1994; Davis et al., 1999). This advantage for small-diameter xylem conduits for evergreen woody plants subject to freezing temperatures may explain the loss of vessels and a return to a tracheid-based vascular system in Winteraceae as indicated by phylogenetic analyses.

Conclusions

Studies of ecology, historical biogeography, and physiology challenge the long-held belief that Winteraceae are an unchanged and relictual angiosperm lineage. Further investigations into the structure and function of Winteraceae morphological features with respect to their natural environments will continue to inform discussions on their evolutionary history.

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ANATOMY OF THE YOUNG VEGETATIVE SHOOT OF *TAKHTAJANIA PERRIERI* (WINTERACEAE)[1]

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ABSTRACT

Material of the rare monotypic genus *Takhtajania* has been examined for anatomy of the young vegetative shoot. Leaf cuticles are smooth, stomata mostly brachyparacytic and free of alveolar deposits common to other Winteraceae. Mesophyll structure is weakly differentiated. Petiole bases are vascularized by three independent traces, each of which may form pairs at the nodal level. Splitting of lateral traces leads to a more or less 5-trace V-pattern at higher levels of the petiole. Young stems are pseudosiphonostelic and have little sclerenchyma. Within the family, *Takhtajania* appears among the least specialized genera, with *Drimys* and *Tasmannia* coming closest histologically. Among other ranalian families, Canellaceae appear to be nearest this level of specialization, mostly on the basis of similarities in stelar and nodal structure.

Key words: Canellaceae, leaf, nodal anatomy, stem, *Takhtajania*, vegetative shoot, Winteraceae.

Phylogenists have long considered Winteraceae to be among the least specialized in the angiosperms (cf. Takhtajan, 1997). Among analyses supporting this conclusion are those based on the presence of vesselless wood (Carlquist, 1983; van Tieghem, 1900), a mixture of sieve-element plastids (Behnke, 1988; Behnke & Kiritis, 1983), plesiomorphic floral structure (Erbar & Leins, 1983; Endress, 1983; Vink, 1988), and paleoantarctic distribution (Smith, 1945). *Takhtajania*, the rarest and most recently recognized genus, has been known only from dried type material. It is isolated from other Winteraceae geographically and morphologically. Only recently has liquid-preserved material been available in support of a detailed study of its morphology and anatomy. The most important questions to be initially considered were (1) what is the place of the genus among its likely neighbors, and (2) what is its level of specialization within the woody Ranales.

The original *Takhtajania* collections were made in Madagascar in 1909 by Perrier de la Bâthie. They awaited examination at Paris (P) until Capuron (1963), finding them to represent a new taxon of Winteraceae, named the collection *Bubbia perrieri*. Later study of the leaves by Baranova led to reevaluation of *B. perrieri* and its recognition as the monotypic genus *Takhtajania perrieri* (Capuron) Baranova & J.-F. Leroy. At the same time, it was placed in a new subfamily Takhtajanioideae by Leroy (1978). The species remains geographically isolated from other known genera of the family.

After repeated attempts over the past 25 years to find more material, Malagasy parataxonomist Fanja Rasoavimbahoaka located a second population of *Takhtajania* in 1994 about 150 km east of the original locality. Then in spring 1997, the new locality was revisited by parataxonomist P. J. Rakotomalaza and Chris Birkinshaw who collected liquid-preserved flowering and vegetative material. For more detailed information regarding the interesting collection history of this taxon, see Schatz (2000 this issue).

At the Missouri Botanical Garden, the preserved materials were divided among several investigators who are contributing observations on wood and reproductive organs. This contribution includes observations on leaf vasculature from clearings and transsections of the leaf lamina and midrib, petiole and nodal anatomy, and other anatomy of the young vegetative shoot.

MATERIAL AND METHODS

Specimens of vegetative and flowering material were collected in the Anjanaharibe-Sud Special Reserve southwest of Andapa in northeastern Madagascar as follows: *P. J. Rakotomalaza et al. 1342*, and *C. Birkinshaw 483*, 14°45′S, 49°28′E, at 1200 m altitude in a perhumid forest with abundant moss and lichens covering the stems.

[1] I thank Thomas K. Wilson, Miami University, Oxford, Ohio, for useful discussions and the use of his laboratory facilities for preparation of some samples. I also thank W. L. Stern and an anonymous reviewer for thorough reviews.

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Young stems and leaves used in this study were field preserved in 50% ethanolic FAA and transferred to 70% ethanol upon arrival at the Missouri Botanical Garden. Leaf clearings were made by an improved method (Keating, unpublished) where specimens were placed in cold (room temperature) 5% NaOH and then microwaved. The 750 watt magnetron was set on defrost cycle, i.e., pulsed power, 2 seconds on/off for three cycles of 20 seconds. This promoted uniform clearing and avoided boiling, which is quite destructive of soft tissues. The NaOH-covered specimens were then placed in a 45°C oven for 48 hours during which time clearing was completed. Specimens were immersed in cold (room temperature) 5.25% sodium hypochlorite (Clorox®) for 30 minutes until the specimen was white. After three gentle tap water rinses, the specimen was soaked in I_2KI (0.2/2.0%) (v/v) in 15% ethanol for two hours until uniformly brown. Without further aqueous rinse, the specimen was changed to 20% $CaCl_2$ solution and then mounted. Within minutes, dark magenta veins contrasted against a transparent background in the cleared specimens.

Cross sections of leaf lamina and midrib, petiole, node, and young stem were made by hand microtome as well as free-hand and mounted in $CaCl_2$. Some sections were mounted unstained in glycerine. Most were stained in cresyl violet acetate, toluidine blue, Schiff's reagent, or I_2KI. Differentiation of these dyes is sharp in the strongly ionic $CaCl_2$ (Herr, 1992; Keating, 1996). Two additional specimens of liquid-preserved young stems with attached leaf bases were paraffin-embedded, serial-sectioned transversely on a rotary microtome at 10 μm, and stained in Safranin-O and Fast Green FCF.

Observations

Shoot Apex

The young stem expands massively within 5–7 mm of the shoot tip causing the apical meristems to appear somewhat embedded among massive petiole bases (Fig. 1A, B). From an early stage, petiole bases show an obvious cicatrice constriction (Fig. 1A). Young primordia form a 2/5 phyllotaxy of petiole bases that show early cell division and expansion, laterally and dorsiventrally. Packing geometry causes petiole bases to remain partially fused at the shoot apex level and to form polygonal shapes (Fig. 1C). The axillary bud is, in two observations, an outgrowth of the adaxial side of the petiole of the 7th primordium (Fig. 1D). No vascular connections could be followed, nor was any bud development visible in the true axil of more mature nodes.

Leaf Surface and Paradermal Anatomy

Leaf outline elliptical (Fig. 2A) with a decurrent base (Fig. 2B). Length/width ratio 4–5:1. Cuticle smooth. Epidermis: cells rounded-polygonal (1–3: 1 l/w) (Fig. 2C). In some areas of older leaves cuticular flanges form thick dividers between cells, 3–7 μm wide. Stomata abaxial only, guard cell pairs with lengths 22–29 μm, widths 18–21 μm, most commonly brachyparacytic with four adjacent subsidiary cells that differ only in position from normal epidermal cells (Fig. 2C). Occasionally ca. 20% of stomata have 5 or 6 subsidiary cells, but remain brachyparacytic with regard to the presence of two epidermal cells always parallel with the guard cells. Mesophyll: cells of adaxial palisade zone appearing compact and shortly lobed with ca. 10% air cavity space. Spongy tissue with numerous air cavities 2–3× as broad as cell diameters. Air space ca. 60%. Venation pinnate, looped brochidodromous. Intercostal areas irregular. Higher-order venation showing incomplete and very irregular areolation (Fig. 2D), 0–6 vein endings per areole (Fig. 2E). Areole sizes: smaller ones ca. 340 μm isohedral; larger ones ca. 2610 × 1248 μm.

Leaf Transsection

Structure weakly dorsiventral (Fig. 3A). Midrib: outline shallowly concave adaxially, deeply convex-rounded abaxially. Cuticle smooth, thickness 5–7 μm (Fig. 3B). Cuticular flanges absent or narrow between epidermal cells, up to 18 μm deep. Epidermis: cells thin and cuboidal on both surfaces. Stomata level with surface. Guard cells small, oblique to and oriented toward the surface in relation to adjacent subsidiary cells, which subtend the guard cells internally (Fig. 3D). Outer cuticular ledge well developed at stomatal opening; internal ledge not present or much smaller. No cuticular alveolar deposits occlude the stomatal openings. Hypodermis generally absent although adaxial-most layer of mesophyll may lack chloroplasts in some areas. Mesophyll differentiation weak. Cells of the palisade zone mostly small, rounded cells in 1–3 closely packed layers, occupying up to 25% of mesophyll, forming a gradual transition to spongy tissue. Spongy cells in lower mesophyll horizontally elongated with irregular long lobes. Chloroplasts most densely packed in adaxial mesophyll cells, gradually becoming more dispersed in abaxial mesophyll. Air cavities: most larger cavities abaxial and aligned over stomata as deep substomatal cavities extending above the center of the mesophyll (Fig. 3A, D).

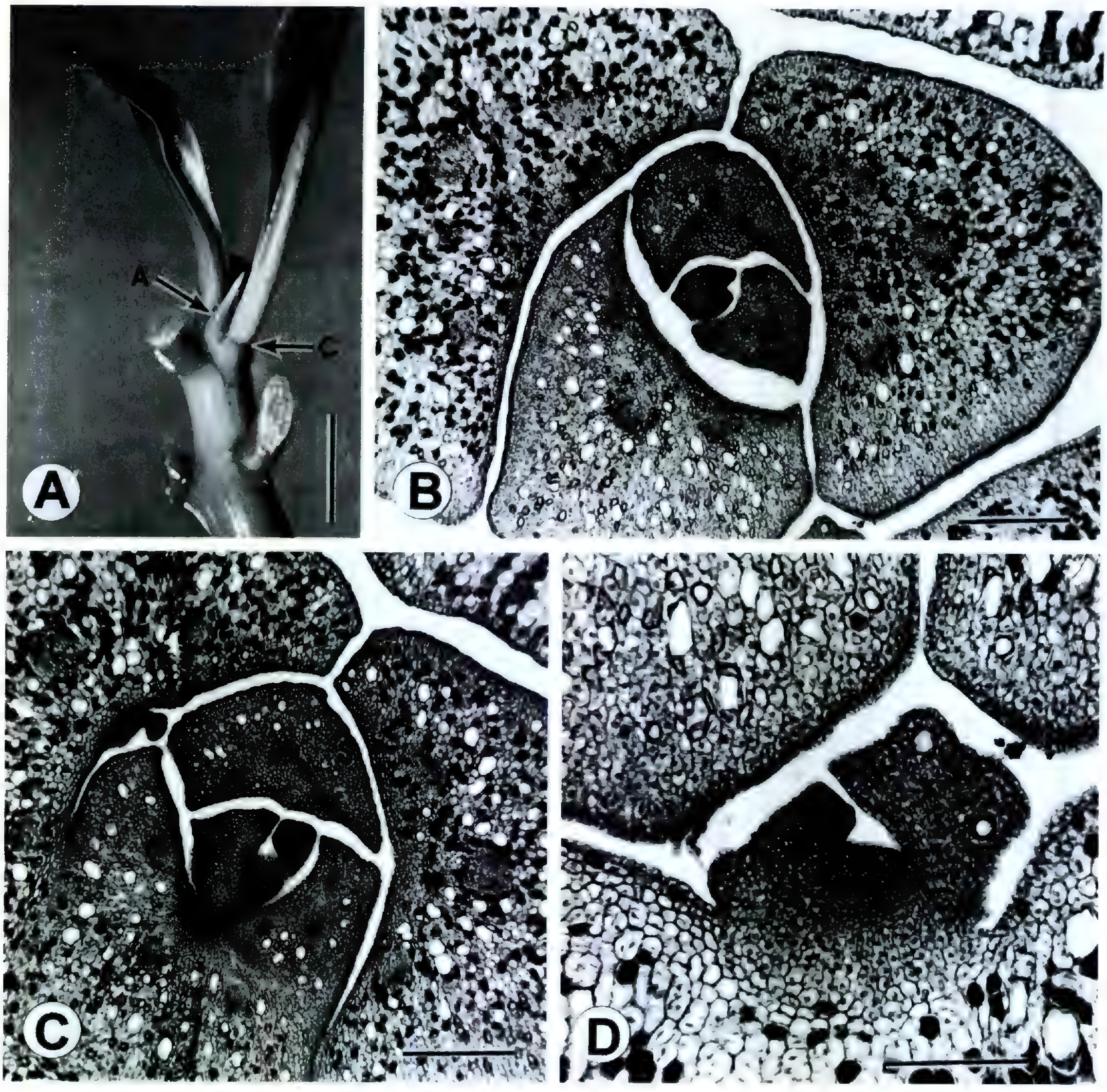


Figure 1. *Takhtajania perrieri* shoot tip and apical meristem region. —A. Young shoot tip showing apical meristem region (A-arrow) and cicatrice constrictions (C-arrow). —B. Transsection of young stem tip, ca. 100 μm distal to apical meristem. Base of leaf primordia determined by packing geometry. —C. Shoot apex at apical meristem level. Bases of several leaf primordia fused as meristem is partially sunken at shoot summit. —D. Shoot apex ca. 120 μm distal to apical meristem. Axillary bud attached to leaf primordium base. Scale lines: A = 1 cm; B, C = 300 μm; D = 150 μm.

Midrib vasculature usually 5 bundles arranged in a V-shape, one abaxial bundle and 2 or 3 lateral bundles on each side. Xylem: largest bundles occur with 10–12 files of metaxylem facing phloem with a convex procambial boundary. Phloem: crescent-shaped strand with weak or no visible differentiation of sieve elements and companion cells. Cells show no alignment or regularity of pattern in cross section. Bundle sheaths a single layer of thin-walled cells, little modified from spongy tissue. Sclerenchyma: lateral-most bundles in midrib have 1–2-layered extraxylary fiber cap outside phloem. Secondary bundles with two lateral fiber strands at phloem and small strand capping xylem. Secretory tissue: slightly enlarged oil cells frequent in mesophyll (Fig. 3C, E). Tannin cells in midrib area in discontinuous row bounding phloem; some dark smaller cells within phloem. Starch grains either common throughout cells of the lamina, or grains mostly in spongy cells and not numerous. In midrib, starch grains packed in ground tissue internally adjacent to xylem points. Grains less common but often well developed in all ground tissue in vicinity of vascular strands.

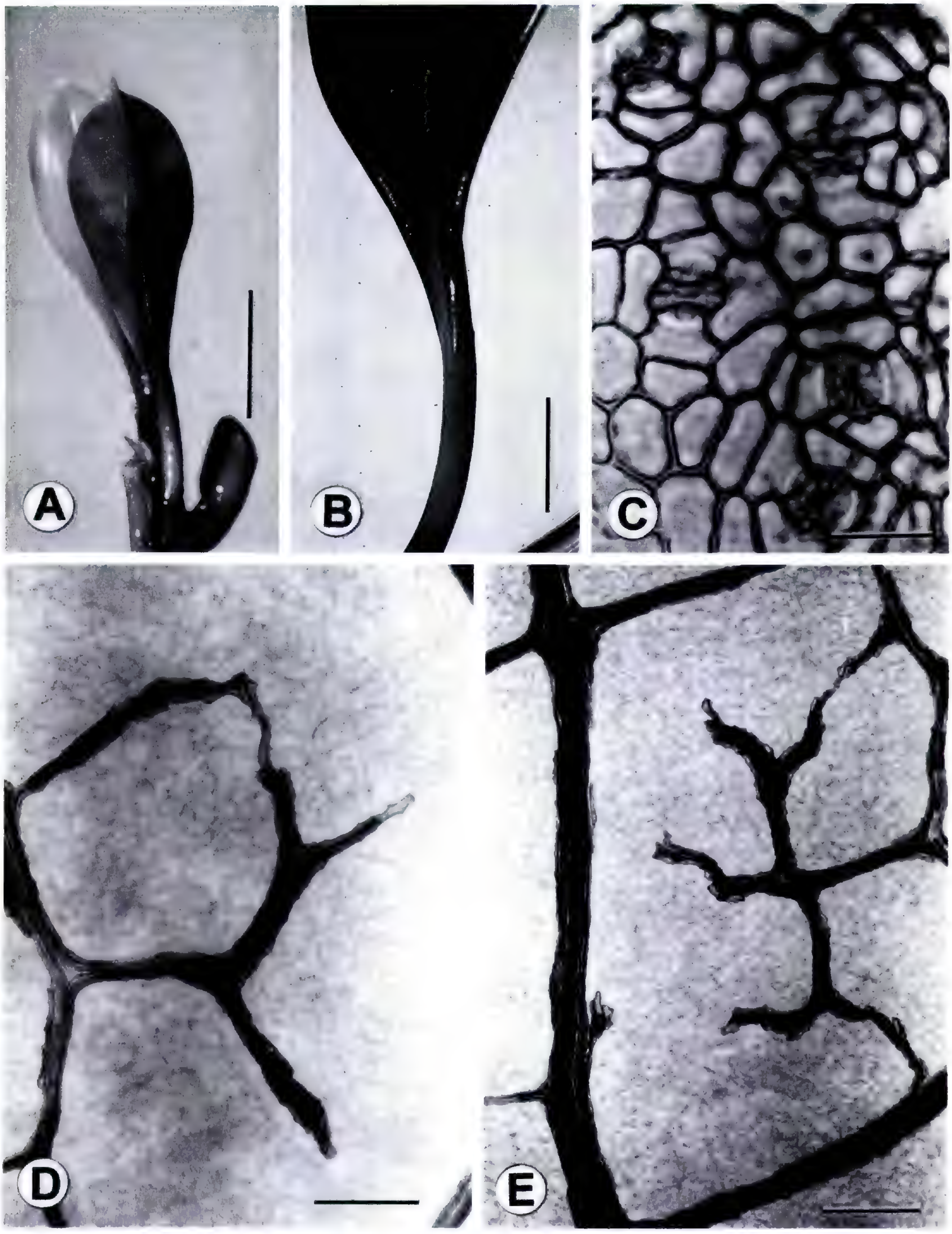
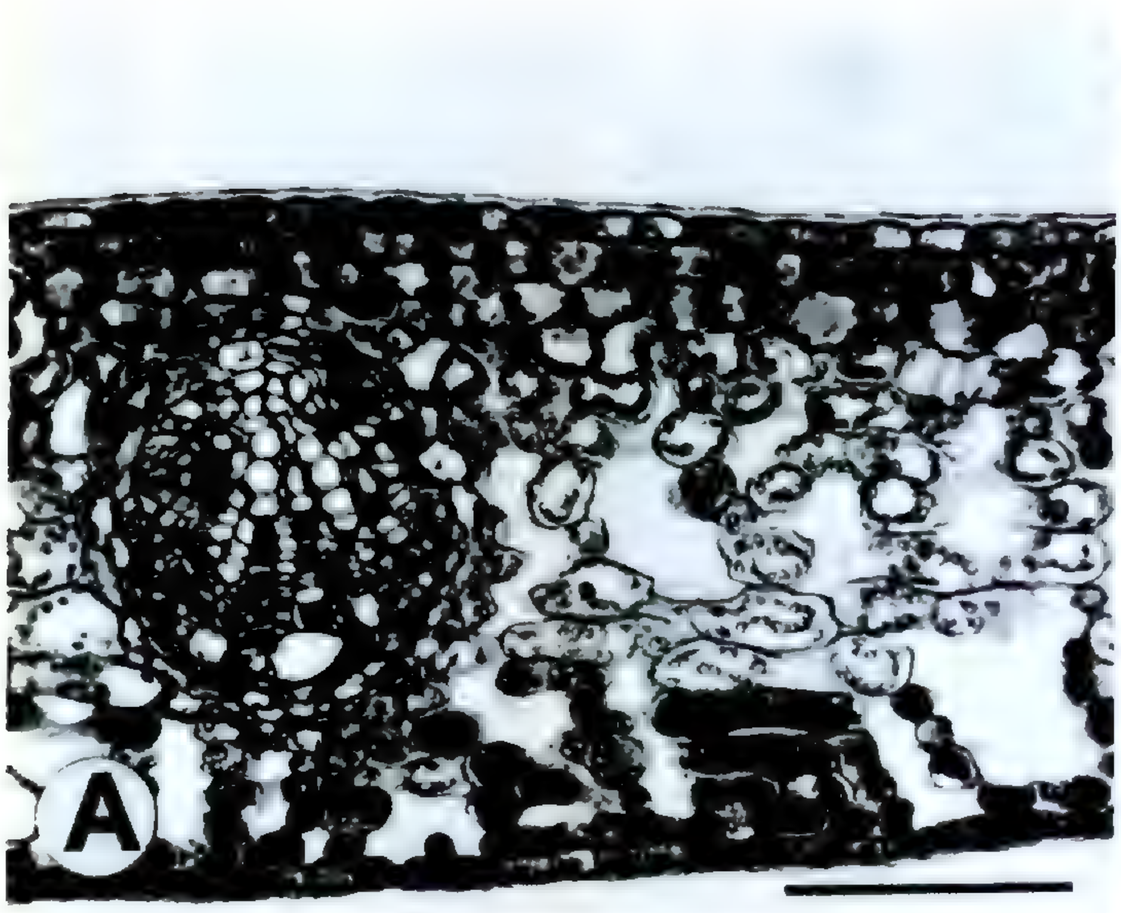
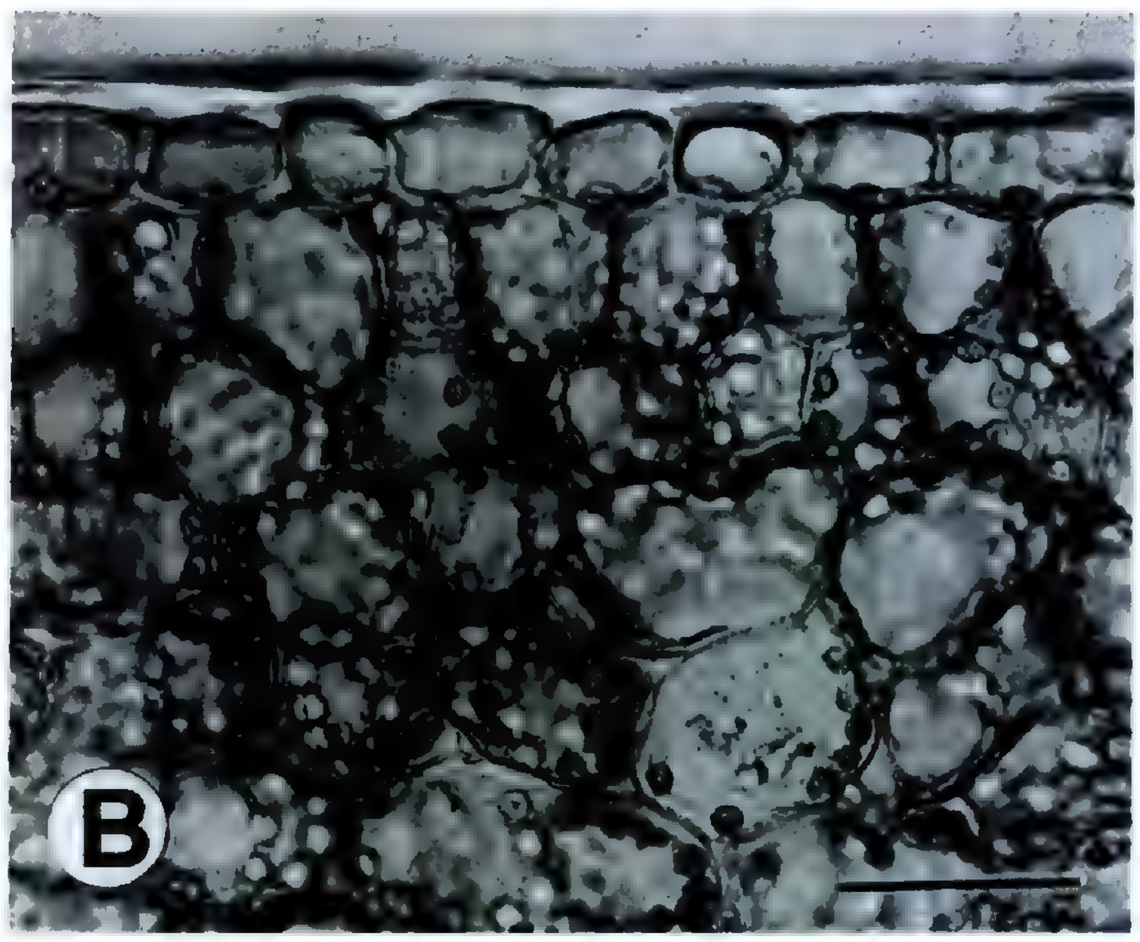
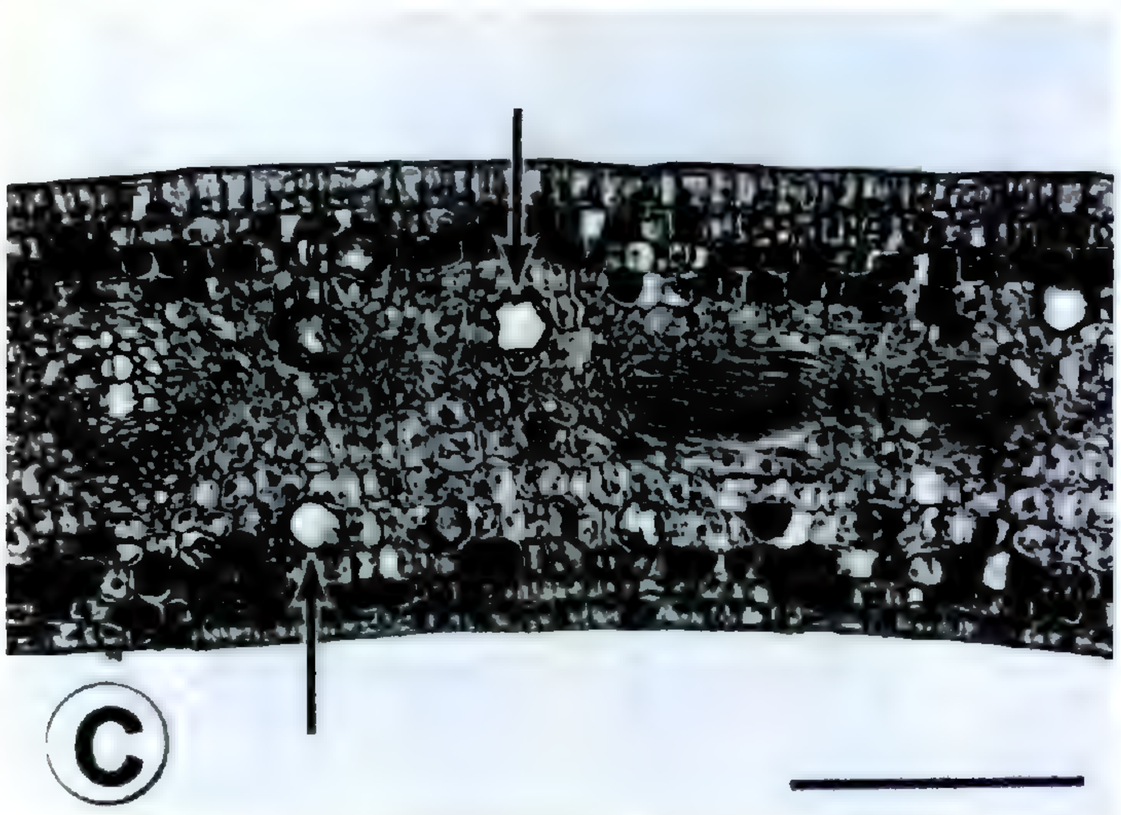


Figure 2. *Takhtajania perrieri* young leaf structure. —A. Pair of young leaves, not actually opposite, at shoot apex. —B. Young leaf, adaxial surface showing decurrent lamina. —C. Abaxial surface of epidermis showing polygonal epidermal cells and stomata. —D. Leaf clearing showing small areole and free vein endings. —E. Leaf clearing showing large imperfect areole and free vein endings. Scale lines: A, B = 1 cm; C = 50 μm; D = 200 μm; E = 300 μm.







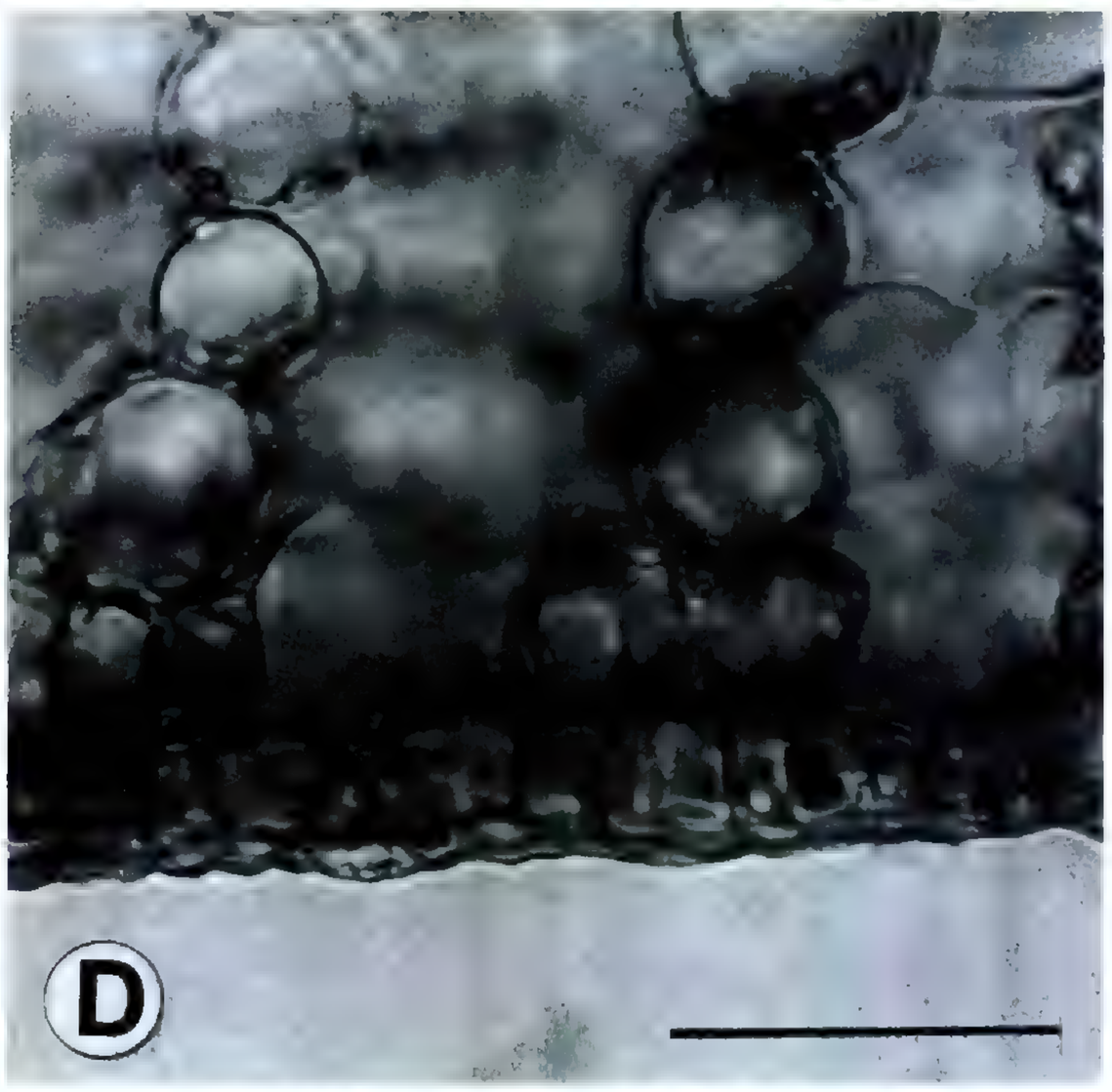


Figure 3. *Takhtajania perrieri* leaf and petiole structure. —A. Lamina transsection (TS) showing compact mesophyll in palisade region and circular vascular bundle. —B. Lamina TS, hand section showing smooth thick adaxial cuticle. —C. Young lamina before mesophyll expansion showing several mature oil cells (arrows). —D. Lamina TS abaxial surface. Stomata with guard cells arranged obliquely to epidermal cells. —E. Young lamina showing young oil cells. —F. Petiole base showing V-pattern of median bundle and divided lateral bundles. Scale bars: A, D = 150 μm; B, C, E = 50 μm; F = 300 μm.

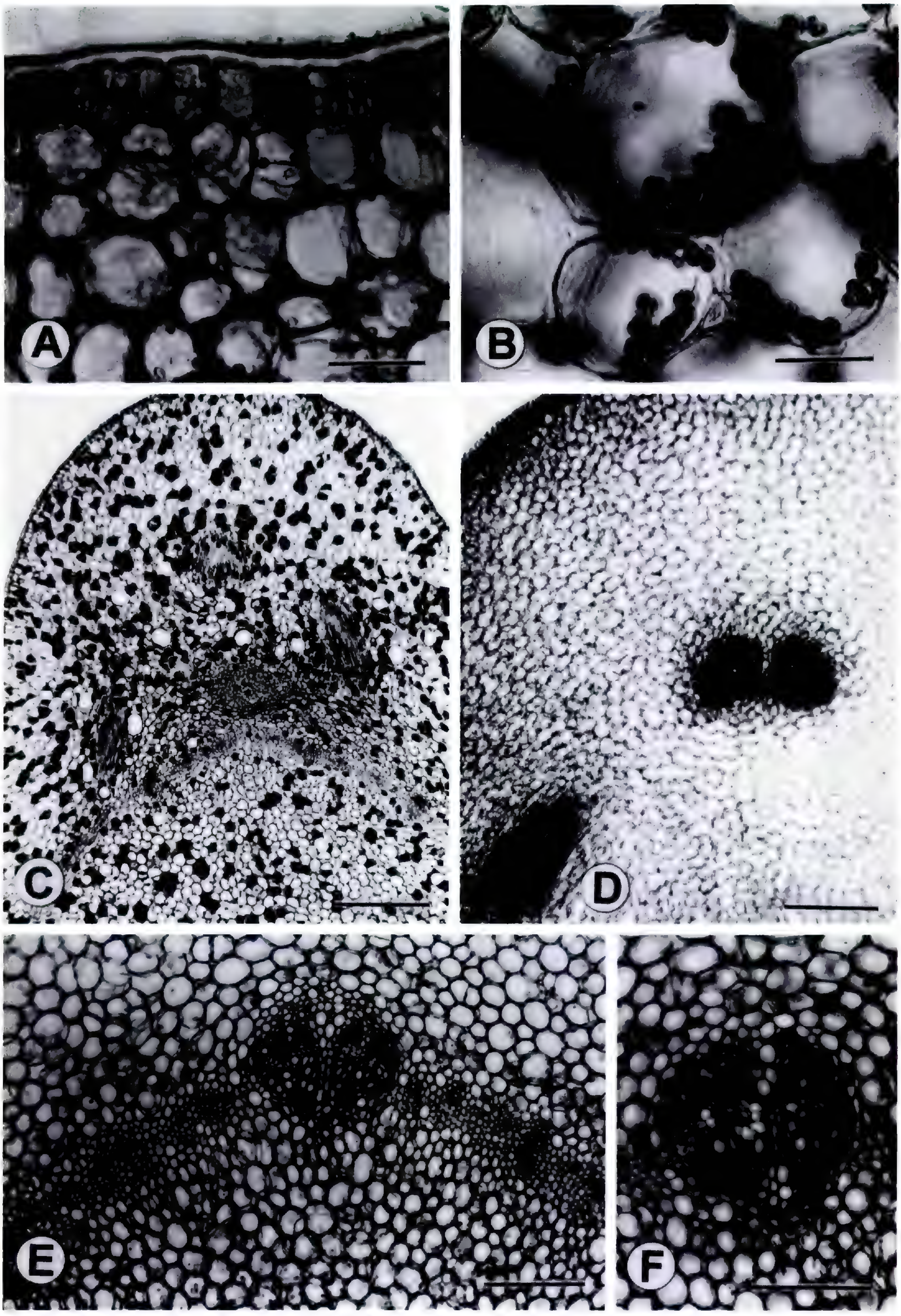


Figure 4. *Takhtajania perrieri* stem and nodal structure. —A. Young stem TS outer cortex, epidermis, and cuticle. —B. Stem TS pith and starch grains. —C. Node showing median trace and two lateral traces. —D. Node TS hand section showing split median trace and one lateral trace. —E. Node TS divided median trace within the stele. Small

PETIOLE TRANSSECTION

Outline: flattened adaxially at base, becoming terete distally. Size: diameter 2.5 mm. Cuticle smooth, thickness 3.6–7 μm. Epidermis: cells small and cuboidal, diameter ca. 22 μm. Ground tissue: cells spheroidal, diameter 26–70 μm, loosely packed but with no organized aerenchyma. Intercellular air spaces at cell corners comprising 10–20% of ground tissue. Venation: 3 to 7 collateral vascular bundles arranged in V-shape (Fig. 3F). Xylem: several protoxylem points lead to organized files of metaxylem, up to three cells wide per protoxylem point. Xylem production ends at straight or broadly convex cambial boundary with hemielliptical phloem strand. Phloem: sieve elements and companion cells in tiers 1–2 cells wide. Tannin cells abundant in ground tissue, these not differentiated from ground tissue except by dark-staining contents. Tannin cells randomly scattered abaxially or in radial rows adaxially. Starch grains common in cells adjacent to vascular bundles.

YOUNG STEM TRANSSECTION AND LONGISECTION

Outline terete. Diameter ca. 1 cm. Cuticle smooth, thickness, ca 3.5 μm (Fig. 4A). Epidermis: cells small and cuboidal. Chlorenchyma: chloroplasts very common in outer cortex. Cortex: ground tissue cells mostly spherical, loosely packed with numerous air cavities. Cells axially elongate, 2–3× cell diameter in transsection. Air space ca. 20% of cortex. Pith cells polygonal and somewhat larger than cortical parenchyma cells; air cavities 5–10% of pith. Vascular bundles collateral. Xylem: distinct protoxylem points at pith boundary but xylem quickly develops cambial growth producing a pseudosiphonostele within 90 μm of pith boundary. Phloem: sieve element arrangement irregular. Companion cells not easily distinguished in cross section. Widest pith rays with slight tendency toward ray dilation in phloem. In longisection, metaphloem sieve element length up to 395 μm. Early secondary sieve element length 215–290 μm. Sieve plates horizontal or 5–10° off horizontal and somewhat broadened. Companion cells short or mostly as long as sieve elements, highly variable in shape and size.

Sclerenchyma: no extraxylary fibers present in small stems. In one sample, ca. 9 mm diameter, occasional single fibers occur around phloem perimeter in inner cortex. In longisection, these single cells overlap and are contiguous for at least longer than 2 cm. In same larger specimen, sclerotic layer occurs subepidermally around perimeter. Sclerotic cells in 1–3 layers, heavily lignified, with up to 20 isotropic wall layers especially well developed on inner and outer periclinal walls. Tannin cells scattered and common in all ground tissue. Starch grains rounded, single or in clusters (Fig. 4B). Grain diameter 9–13 μm. Grains common throughout pith and in sheath of 5–10 inner cortical cell layers.

STEM–NODE–LEAF CONTINUUM

Stem outline in the vicinity of the node is irregularly elliptical. In the young stem, the vascular procambium appears to be a nearly continuous circle with maturation of proto- and metaxylem only observed in what will become leaf traces. A characteristic cut through the node of a stem with a young active cambium shows three non-adjacent traces vascularizing the leaf base, i.e., the node would be called three-trace, tri-lacunar (Fig. 4C) using the original siphonostele paradigm begun by Jeffrey (1898) and carried into dicot nodal anatomy by Sinnott (1914). However, the two specimens observed here show some intriguing differences. The older stem sections show most leaf traces appearing double, that is, there are two proto-xylem points causing the development of two paired traces. They may cause a node to appear 2:2:2, 2:2:1, or 1:2:1 (Fig. 4D, E, F). In the younger stem pieces, which were paraffin-sectioned at 10 μm, this observation was confirmed less frequently. Diagramming the vasculature through several nodes showed mostly late-appearing bifurcations of larger bundles.

Nothing was found to indicate that the individual leaf traces, especially the median trace, have components that occur in different sympodia of the vasculature. In this material, leaf traces were well defined through at least four nodes before entering a leaf base. New leaf traces appeared to arise from undifferentiated interfascicular procambium. More material, sectioned at closer intervals, will be needed to identify vein sympodium branching patterns. Following the leaf base distally from the node, the three traces are observed to relate in a V appearance, whether or not they appeared as double at

←

incipient traces visible within the procambial cylinder. —F. Node TS showing divided lateral traces at level of procambial cylinder. Scale bars: A, B = 50 μm; C, D = 300 μm; E = 200 μm; F = 150 μm.

the nodal level. Distally, in the leaf base, the two lateral traces bifurcate to form two pairs of lateral traces. Using Sugiyama's (1979) notation the *Takhtajania* node can be most usually figured (moving distally into the petiole) as L-M′M-L ⇒ L-M-L ⇒ L′L-M-L′L. In the base of the petiole and continuing up to the lamina, the resulting five traces form a marked V of closely associated vascular bundles with the median trace forming the base of the V.

Axillary buds do not appear among the first 4–5 leaf primordia. They occur removed from the axil, high on the adaxial side of young petioles (Fig. 1D). No vascular connections to axillary buds were detected in this material.

Discussion

THE LEAF

Leaf venation is typical of other ranalian families with elliptic leaves with entire margins. The pinnate, festooned brochidodromous structure, accompanied by imperfect and variable areole structure, produces a low first-rank leaf (cf. Hickey, 1977: 158).

Metcalfe (1987) noted much variability in cuticle texture and sculpturing in Winteraceae, and *Takhtajania*'s smooth, unsculptured cuticle seems diagnostic within the family. Bongers (1973) noted that leaf cuticles of *Takhtajania* and some *Drimys* species lack the alveolar layer present in all other Winteraceae. However, Baranova (1972) noted that "Group 2" Winteraceae, *Bubbia, Belliolum, Pseudowintera,* and *Zygogynum,* also have ordinary (= non-alveolar) or more or less grainy cuticles. Existing data are not a useful guide to relationships until comparable observations can be made across the genera. The known leaf venation trends are not sufficiently refined for one to offer an opinon on whether *Takhtajania* is more or less specialized than other winteraceous species.

Leaf surface observations do not confirm Baranova's (1972) report of the existence of mostly anomocytic stomata in *Takhtajania*; instead, stomata are mostly brachyparacytic as is true of other winteraceous genera. This removes a major argument for subfamily-level segregation of the genus. Also, stomatal apertures are said to be occluded with alveolar material (Metcalfe, 1987; Bongers, 1973; Baranova, 1972), but this is not true in *Takhtajania* or in *Drimys* (*Tasmannia*) *piperita* Hook. (Bongers, 1973).

In genera studied thus far, including *Takhtajania,* mesophyll in the family is reported to be not clearly differentiated into palisade and spongy mesophyll, e.g., *Exospermum* (Carlquist, 1982) or *Zygogynum* (Sampson, 1983). This weakly bifacial structure is consistent with a relatively low placement of this leaf structure among the Ranales. Bailey and Nast (1944b) noted that thicker, more coriaceous leaves in *Drimys* have large sclereids in the mesophyll between vascular bundles, and Rao and Das (1979) also reported nests of sclereids in the family. By their absence, *Takhtajania* shows simpler structure. In most Old World genera of Winteraceae, all foliar vascular bundles are commonly ensheathed by a slender ring of thick-walled sclerenchyma cells. This was not observed in *Takhtajania.*

THE NODE

Observations on nodal anatomy are not as systematically powerful as they could be, partially due to persistent use of the siphonostele paradigm (see review by Beck et al., 1982), partially to unsolved theoretical concerns regarding the reality of cauline vasculature, and partially to the difficulties in relating nodal traces to stelar vasculature. At least for three-trace ranalian genera showing open architecture, the following discussion predicates that vascular patterns are best recognized as a cylinder of eustelic sympodial bundles of varying number. Followed from a proximal to distal direction, all of these bundles become leaf traces at regular intervals. In terms of their origin, all so-called cauline bundles are first identified as leaf traces. They probably connect basipetally to existing leaf trace sympodia.

As seen from the contiguous older nodes and two short pieces of stem tip available in the present study, nodal structure places *Takhtajania* among Benzing's (1967) trilacunar ranalian families. Benzing noted that nodes in *Bubbia* sp., *Drimys colorata* Raoul, *D. winteri* J. R. Forst. & G. Forst., *Canella alba* Murray, and *Warburgia ugandensis* Sprague are so similar that they can be described as one. All five species showed a 2/5 phyllotaxy, which is also confirmed in *Takhtajania.* Benzing's specimens showed distinct helicoid sympodia where every 5th leaf is supplied by a median trace from the same sympodium. This seems possible but could not be quite confirmed from currently studied *Takhtajania* material. The relationship of 3 traces vascularizing a leaf to similar sets of traces above and below that node appears somewhat unpredictable and irregular in the available material (Fig. 5).

About 5 mm below the apical meristem, in a region of closely spaced nodes, the vasculature of the young stem forms a eustele consisting of precociously matured xylem strands, here interpreted

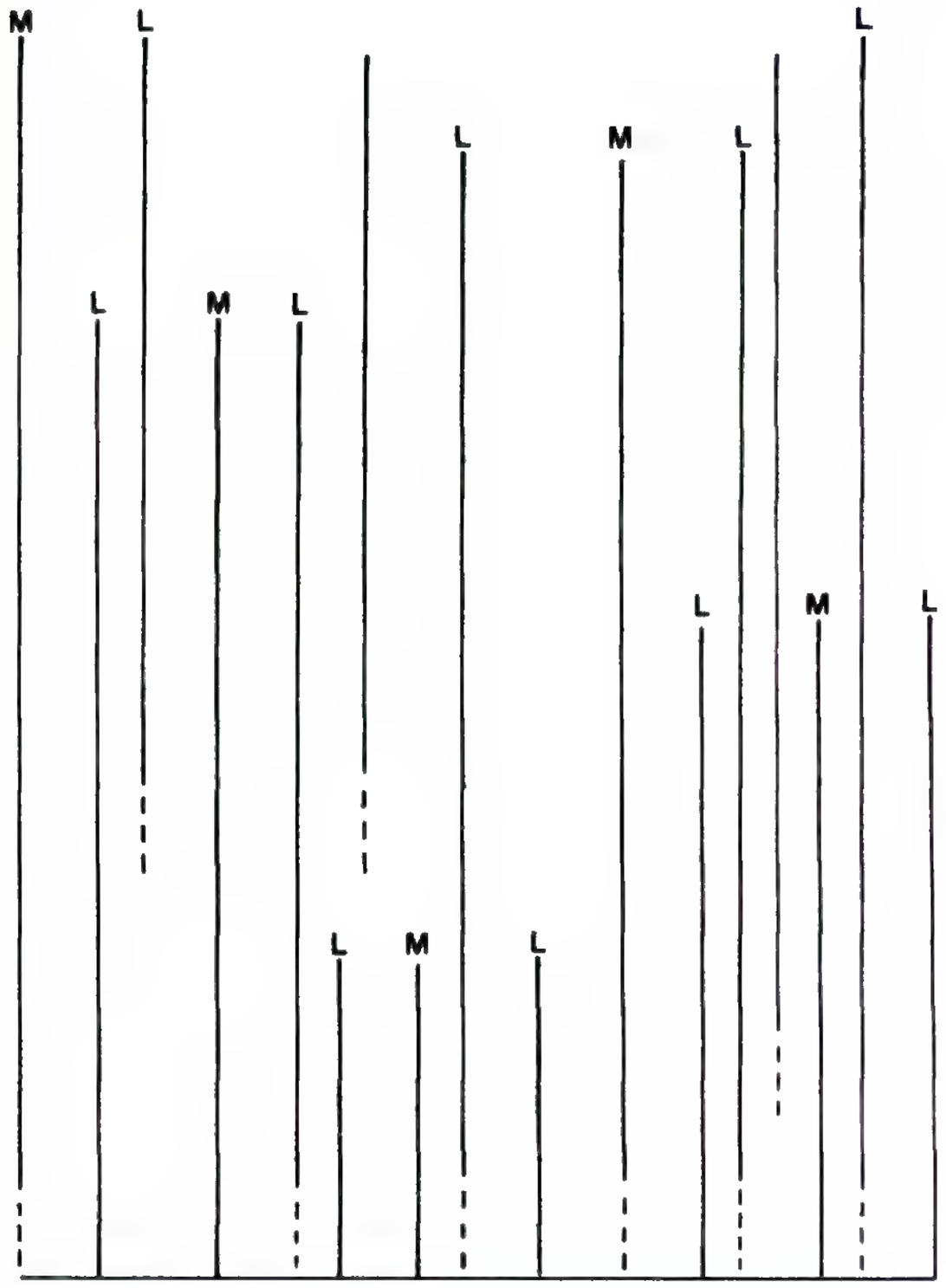


Figure 5. *Takhtajania perrieri*: diagram of shoot vascular pattern through five nodes, reconstructed from 10 μm serial sections. Eustelic bundles appearing as solid lines, each eventually becoming a leaf trace. Incipient traces occurring entirely within cambial zone shown as broken lines. M = median leaf trace; L = lateral leaf trace. Other incipient traces, 2–5 between larger traces, are not shown. Leaf trace bifurcations occurring at various levels also not shown.

as leaf traces, embedded in a cylinder of procambium. Usually, the clearly defined vascular bundles occur in pairs or threes, rather than as singles. The vascular diagram for *Takhtajania* (Fig. 5) shows that lateral and median leaf traces arise independently. All of the closely associated trace pairs do not arise from different sympodia as far as can be followed. All are superficial bifurcations arising distal to their identity as single incipient traces in the procambial cylinder.

An urgent need is the diagramming of the vasculature pattern from sections in the 1–3 μm range to see if the type of sympodial arrangement of terminal traces and renewal branches drawn by Benzing (1967) for a number of ranalian families can be confirmed. From the three short stem pieces available for this study, such an integrated pattern cannot be drawn.

As noted above, several relevant studies have to be translated from the cauline vasculature concept. In Bailey and Nast's (1944a: 215) study of the Winteraceae, they suggested that foliar bundles arise from "interfascicular parts of the hypothetical, cauline, primary vascular cylinder." Also, Beck et al. (1982: 795) stated, "There are 5 protoxylem strands in *Drimys winteri* that apparently represent axial bundles . . . ." Both of these conjectures cannot be related to the present observations. In *Takhtajania*, only leaf traces can be discerned when following vasculature through 4–5 nodes. There are no sets of 5 protoxylem strands at any level.

Beck et al. (1982: 795) also stated that, while there is much "variation in the mode of lateral trace production in *D. winteri*, one lateral trace typically diverges from the same axial bundle as the median trace, the other lateral may, ultimately arise from an adjacent axial bundle." No such pattern in *Takhtajania* can be observed or interpreted this way. In a similar line of reasoning, Howard (1974) noted that some workers refer to cauline bundles as those that persist in the stele through several nodes. This definition appears somewhat arbitrary since leaf traces, followed through several nodes, are still leaf traces, at least in these ranalian genera with open vasculature.

Nothing resembling the above scenario occurs in *Takhtajania*, nor does it agree with Benzing (1967). In that work, it can be seen that the essence of vascular sympodia is that all so-called cauline bundles are leaf traces, and the number of nodes along the way between a bundle's origin and its orientation into a petiole base does not change this definition. It would help if trace architecture of the ranalian shoot were interpreted consistently in terms of the primary vascular system in order to avoid artificial concepts and terminology (Esau, 1965).

The present observations of a pair of traces related to the median gap are a first report for Winteraceae, nearly fulfilling Takhtajan's (1964, 1969, 1991) conjecture of a hypothetical nodal type, which he believed to be ancestral for the angiosperms. But Takhtajan's prediction of a 1:2:1 set of traces varies from *Takhtajania*'s potential for having pairs at the lateral gaps also (2:2:2, 1:2:2). Swamy (1949) demonstrated pairs of bundles present in the cotyledonary node of *Degeneria* (1:2:1) and *Magnolia grandiflora* L. (pairs of median bundles and split laterals), but those taxa have multilacunar mature nodes. Observations by Marsden and Bailey (1955) on *Clerodendrum*, and by Bailey and Swamy (1949) and Dickison and Endress (1983) on *Austrobaileya* described double leaf traces where the components arose from independent portions of the eustele, a condition not found in *Takhtajania*. Other observations by Canright (1955) on Magnoliaceae and by Ozenda (1949) on *Liriod-*

endron, *Uvaria*, and *Anona* described three-trace or multitrace nodes where the median traces bifurcate or branch, tendencies also absent in *Takhtajania*.

In Sugiyama's (1979) broad study of Magnoliales nodal anatomy, *Drimys* (*Tasmannia*) *piperita* was diagrammed. Its three-trace node has a median trace that produces two small lateral traces which then degenerate: L-M-L ⇒ L-mMm-L ⇒ 'LL-M-L'L. In *Takhtajania* there are no aborted lateral median traces, but, otherwise, patterns found in *Tasmannia* are closer than those in most other genera to the pattern found here. If one considers multiple independent nodal traces to represent the least specialized condition for the angiosperms, as do Ozenda (1947), Sugiyama (1979), Neubauer (1981), and I, then the Winteraceae are not close to the angiosperm basal lines in this character. Finally, as noted above, because of the different assumptions brought to past studies in nodal anatomy, early trends of specialization in the Winteraceae will remain tentative until one anatomical study across the family generates descriptions based on one current paradigm.

PETIOLE VASCULAR PATTERNS

Superimposed on the basic three-trace architecture, several genera of Winteraceae develop quite complex petiole vasculatures. Bailey and Nast (1945) proposed two independent, plausible trends of specialization in the *Drimys* node: (1) three strands leading to numerous derivative bundles, and (2) three strands fusing to form a single vascular arc in the petiole. The three traces found in *Belliolum*, *Bubbia*, *Exospermum*, and *Zygogynum* divide to form three abaxial bundles and "numerous smaller bundles irregularly arranged in an adaxial position" (Metcalfe, 1987: 6). This seems to be a development parallel with Bailey and Nast's (1945) first *Drimys* pattern. Likewise, Metcalfe (1987: 6) described the bundles as being "amphicribral, hippocrepiform or appearing as divided bundles with their xylem units facing one another." Carlquist (1982: 281) characterized such a pattern in *Exospermum stipitatum* (Baill.) Tiegh. petioles and midribs as having "peculiar circles of bundles" as well as nests of sclereids in the mesophyll, both characters not found in *Takhtajania*.

Dehay and Ghestem (1969) illustrate distal petiole vasculature for three genera of Winteraceae. *Exospermum*, with its circular or semicircular traces in an irregular pattern, and *Zygogynum*, with numerous traces in three layers, are both quite distinct. *Bubbia*, with its simple V of 7 bundles in the petiole, as well as some species of *Drimys*, appears closest to *Takhtajania*. Such a pattern fits close to the base of Bailey and Nast's (1945) proposed trends for the 3-trace node.

THE STEM

Stem transsectional histology is quite simple. The uniform ground tissue of the cortex and pith is unexceptional. The early development of interfascicular cambium quickly produces an uninterrupted cylinder of cambial derivatives. This was termed the pseudosiphonostele by Bailey and Nast (1948), and the condition is common in woody Ranales. On the pith side of this continuous cylinder, the protoxylem is circumferentially discontinuous. In *Takhtajania*, secondary xylem accumulation initially remains limited as the young stem retains a succulence through the 1 cm diameter stage, the largest material available in this study. (See Carlquist, this volume, for details regarding secondary vasculature development.)

One unusual histochemical feature involves tissue lignification. In the presence of metachromatic dyes in combination with the highly ionic calcium chloride mountant (see Herr, 1992; Keating, 1996), tissue sections of most higher dicots and monocots show marked differentiation of lignified tissues from surrounding ground tissue and phloem. In *Takhtajania*, the lignification process appears to be poorly bounded, spreading beyond the limits of xylem, fiber walls, and cuticles. Using several detection systems (cresyl violet acetate, Toluidine blue, Schiff's reaction, iodine-potassium iodide, or phloroglucinol) few purely cellulosic-hemicellulosic walls were found. There seems to be at least a modest amount of lignification on all cells except sieve elements.

SPECIALIZATION AND RELATIONSHIPS OF *TAKHTAJANIA*

Takhtajania lacks the complexity of histology found in other genera of Winteraceae that have been studied in detail. Its lack of cuticular ornamentation and alveolar stomatal plugs, lack of sclereid development and sparing formation of extraxylary fibers, absence of other histological specializations, as well as its geographic isolation suggest that the plant should be interpreted as plesiomorphic and not particularly close to other winteraceous genera. This is consonant with Vink's (1988) proposed relationships. In terms of relative specialization, it would appear that *Drimys* (which shows quite variable structure) and *Tasmannia* are likely closest neighbors to *Takhtajania*.

On the basis of their analysis of *rbc*L nucleotide sequences, Qiu et al. (1993) suggested that the

Magnoliidae have five major lineages with Magnoliales and Nymphaeales being the most derived. Further, within the Magnoliales, they found Canellaceae and Winteraceae appearing closely related. Conclusions by Wilson (1965) and Benzing (1967) supported recognition of similarities between these families based on comparative anatomy. However, characters in the Canellaceae are variously more and less specialized. Advancements include having distinctively different floral morphology and anatomy, particularly fusions in the androecium (Wilson, 1966). Vascular structure differences include the presence of vessels, considered more specialized than the vesselless condition. On the other hand, sieve elements of Canellaceae have highly oblique to vertically oriented end walls with multiple sieve areas (Wilson, 1965) and are much less specialized than those cells found in *Takhtajania* and other Winteraceae (Zahur 1959).

Some characters are compatible but inconclusive. Sieve element size ranges are about the same in both families (Wilson, 1965). Observations on sieve-element plastids (Behnke, 1988) record the two families as being variable with overlapping P-protein types. Wilson (1965) encountered the same difficulties in attempting to trace the obscure origins of nodal architecture in the young stem of *Canella* such as occur in *Takhtajania*. Bailey and Nast (1945) emphasized the relative isolation of Winteraceae when they noted that, while variation within the family is wide, there is no overlap with other ranalian families investigated. In this context, Canellaceae could be most closely related.

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FLORAL STRUCTURE OF *TAKHTAJANIA* AND ITS SYSTEMATIC POSITION IN WINTERACEAE[1]

Peter K. Endress,[2] Anton Igersheim,[2] F. B. Sampson,[3] and George E. Schatz[4]

ABSTRACT

Floral structure of *Takhtajania perrieri*, the sole species of Winteraceae in Africa/Madagascar, was studied and compared with that in other Winteraceae. Floral organs are in more or less regular whorls, starting dimerous in the outer perianth region and changing to tetramerous and pentamerous in the inner perianth region and androecium. The innermost androecium whorl is often incomplete. The outer tepal pair is congenitally united, and pairs two and three are postgenitally united in their overlapping area in bud. At the transition from the female to the male phase of anthesis the club-shaped stamen filaments are inflated by cell enlargement, accompanied by starch consumption. The dimerous nature of the unilocular gynoecium is confirmed. The normal linear placentae of the two carpels are oblique to almost horizontal, and therefore the gynoecium does not conform to a usual paracarpous gynoecium with parietal placentation. The ovules are larger than those in other Winteraceae. However, the total evidence of floral features clearly shows the position of *Takhtajania* in the Winteraceae. Within the family it fits best in the *Pseudowintera/Zygogynum*-clade, which is sister to the *Tasmannia/Drimys* clade.

Key words: androecium, floral anatomy, floral morphology, gynoecium, perianth, *Takhtajania*, Winteraceae.

Takhtajania perrieri (Capuron) Baranova & J.-F. Leroy is the sole surviving species of Winteraceae in the Madagascan/African region. It achieved notoriety because of its strange bicarpellate but unilocular gynoecium, which is unique for Winteraceae, a feature that was noticed only 70 years after the discovery of the plant in Madagascar (Leroy, 1977, 1978). For almost 90 years the plant was known only from the type collection of 1909, which contained only scarce floral material, and it was thus thought to be possibly extinct. The rediscovery (re-collected in 1994 and determined in 1997; Schatz et al., 1998) offers the possibility for detailed studies of the disputed floral structure and a comparison with the other genera of Winteraceae.

Since the unusual gynoecium structure of *Takhtajania* has puzzled botanists, it seems appropriate to give a short introductory survey of the previous interpretations. In the original description of *T. perrieri*, the gynoecium was described as unicarpellate (Capuron, 1963). Because of the small floral involucre and the apical anthers with subhorizontal thecae, Capuron (1963) associated the plant with *Bubbia* and placed it into that genus as *Bubbia perrieri*. Baranova (1972) later found that the leaf epidermis of the plant differed from other Winteraceae. It was her suggestion that it could be a separate genus that prompted Leroy (1977, 1978) to restudy the flowers (see also Leroy, 1993). To his surprise, he found the gynoecium to be bicarpellate, syncarpous but unilocular. This was at first questioned by Tucker and Sampson (1979), because its external shape scarcely differs from single carpels of some other Winteraceae. However, Vink (1978) confirmed its bicarpellate nature, but interpreted the two longitudinal furrows of the gynoecium as being dorsal in each carpel (because they alternate with the placentae) and not lateral, as Leroy (1977) contended. This was later also accepted by Leroy (1980) and Deroin and Leroy (1993).

Study of the scarce floral material of the type specimen concentrated on the puzzling gynoecium, whereas the other floral organs received less attention. The aim of the present study is thus to provide

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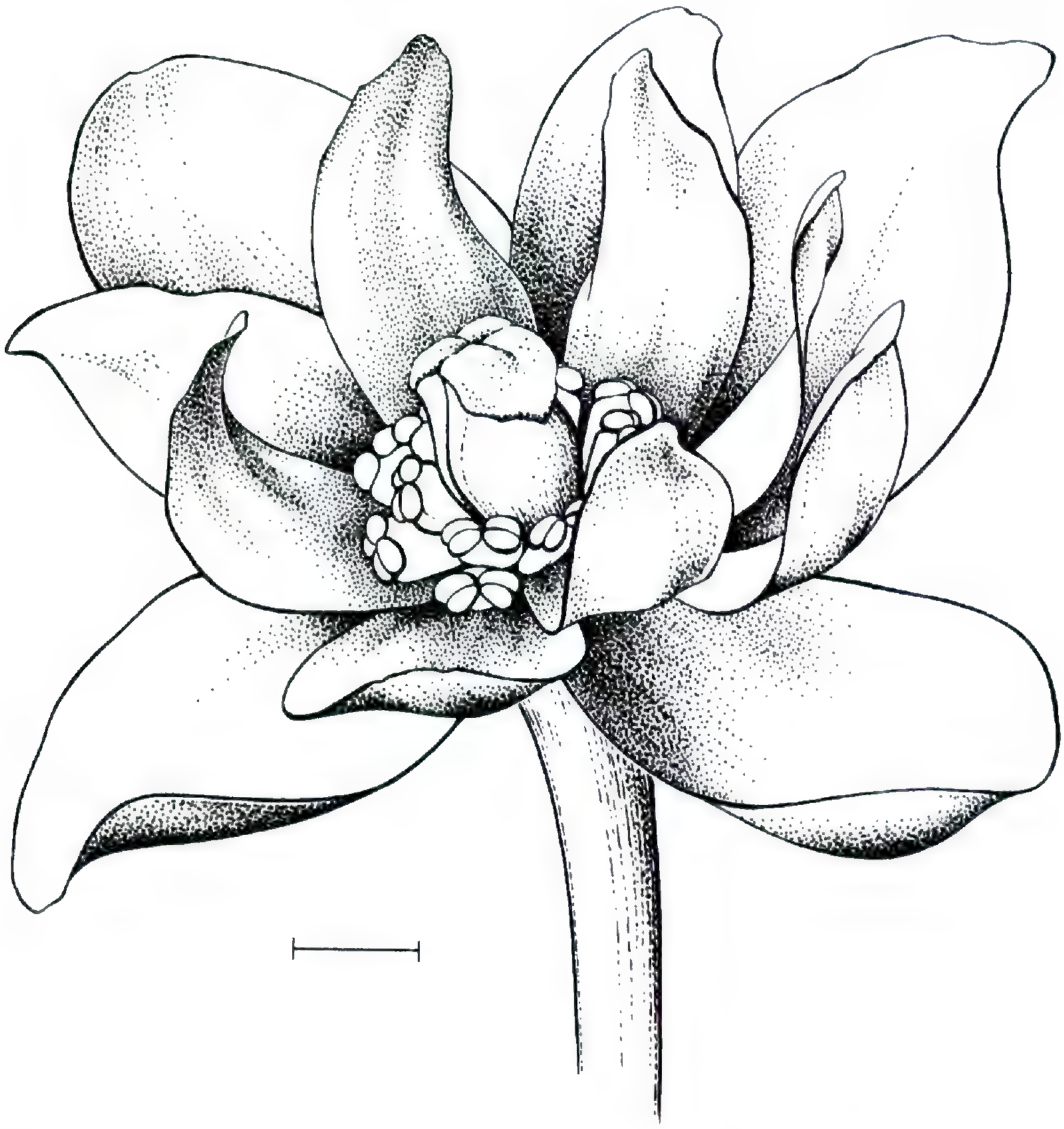


Figure 1. *Takhtajania perrieri.* Anthetic flower at female stage. Scale bar = 2 mm.

a more detailed description of the floral morphology and anatomy of *Takhtajania* and to compare it with that of other Winteraceae, especially the putatively basal *Tasmannia* and *Drimys.*

Materials and Methods

Flowers fixed in FAA were used from the species and collections listed in Appendix 1.

Some of the flowers were embedded in Paraplast, sectioned with a rotary microtome at 10 μm, and stained with safranin and astrablue. Some of the flowers were embedded in Kulzer's Technovit 7100 (2-hydroxyethyl methacrylate), sectioned at 6 μm or less, and stained with ruthenium red and toluidine blue (for details of procedure, see Igersheim & Endress, 1997). For scanning electron microscope (SEM) studies, the specimens were dehydrated in ethanol and acetone and subsequently critical-point dried. The dried specimens were mounted on aluminium stubs and sputter-coated with gold. The confocal microscope photographs were taken by G. K. Rickards, School of Biological Sciences, Victoria University of Wellington, from a gynoecium (of a flower bud immediately before anthesis) cleared in 5% KOH at 40° for approximately 3 days and stained with ethydium bromide (1 microgram per ml).

Phylogenetic analysis was performed with PAUP* vers. 3.1.1, using the heuristic search option, algorithm TBR (tree bisection-reconnection branch swapping), and MULPARS (retention of all equally parsimonious trees) in effect. Consensus trees of shortest trees were gained by reweighting with a rescaled consistency index (CI) of original shortest trees.

Results

Anthetic Flowers

The flowers of *Takhtajania* are ca. 1.5–2 cm across and have spreading red perianth parts at anthesis (Schatz et al., 1998) (Fig. 1). From the behavior of the stamens (see below) they are probably protogynous.

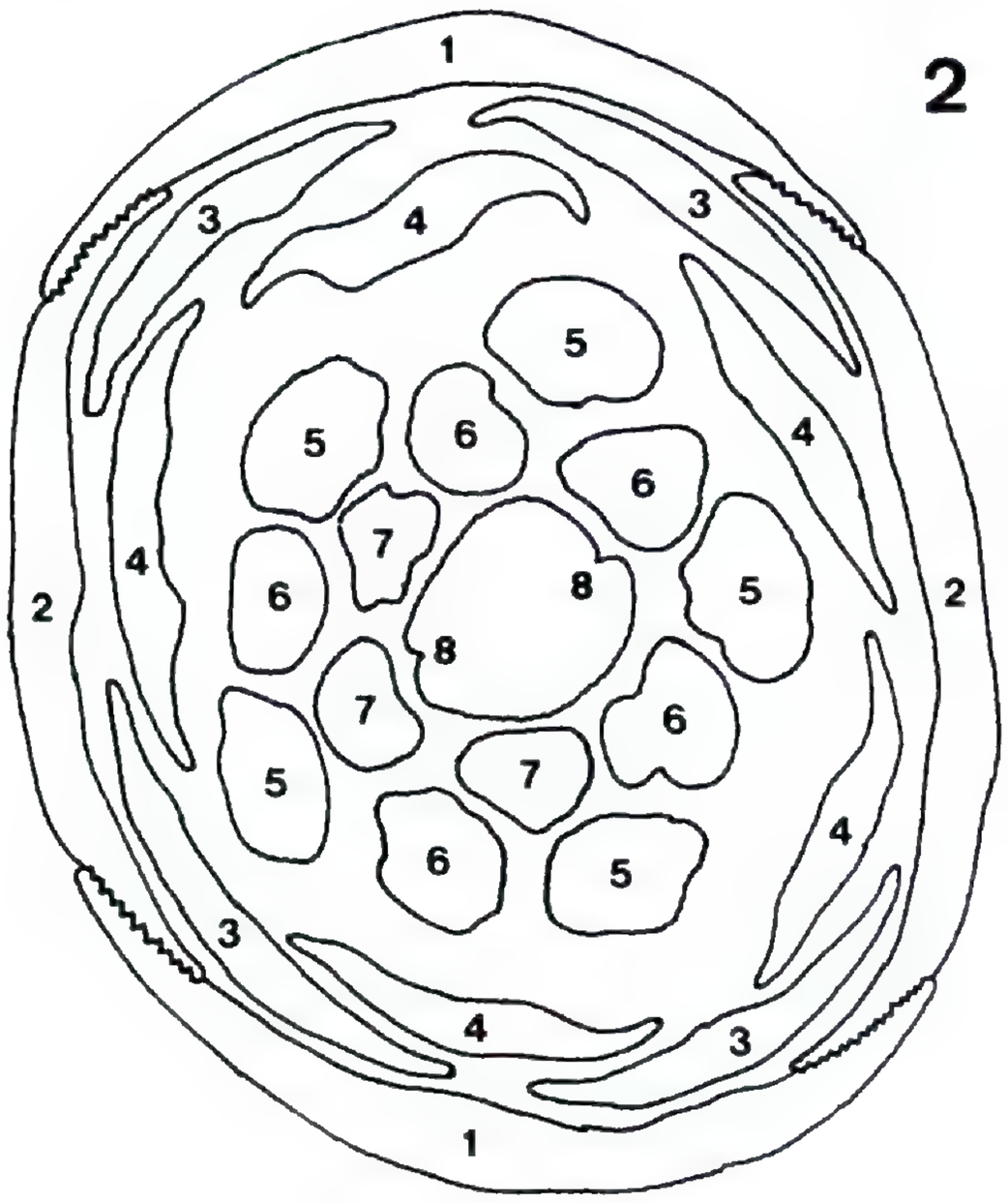


Figure 2. *Takhtajania perrieri.* Floral bud, transverse section; the numbers in the floral organs indicate whorls (1–4: tepals, 5–7: stamens, 8: carpels; the tepals of the involucre are not drawn, because they appear only in sections of the floral base; the tepals of whorl 1 and 2 are postgenitally fused in the overlapping area: marked by zigzag line; whorl 7 is one-sided). Scale bar = 0.5 mm.

NUMBER AND PHYLLOTAXIS OF FLORAL ORGANS

Floral phyllotaxis in Winteraceae is commonly irregular. There have been different attempts to derive phyllotaxis of particular groups from a spiral or whorled pattern (see discussion). In *Takhtajania* only scarce herbarium material has previously been studied, and it is not surprising that in each of the three publications that dealt with the arrangement of floral organs there is a different interpretation of floral phyllotaxis and merosity. These include: (1) outer six perianth parts decussate, stamens in three 4-merous whorls, one carpel (Capuron, 1963); (2) outer six perianth parts decussate, inner organs spiral, two carpels (Baranova & Leroy, in Leroy, 1978); (3) perianth parts in four 3-merous whorls, stamens in three whorls, the two outer ones 6-merous, the inner one 4-merous, two carpels (Vink, 1978).

We studied transverse microtome sections of eight floral buds close to anthesis to determine phyllotaxis and organ number; an example is illustrated in Figure 2. Organ position is somewhat unstable. However, there is a more or less whorled pattern with changing merosity from the outside to the center of the flower. The outermost floral organs are in dimerous alternating whorls. Then there is a switch to 4-merous alternating whorls. The result is that after a 2-merous whorl there appear two instead of one organ in each alternating position, and thus there are double positions instead of single positions (for discussion of double organ positions in flowers, see Endress, 1987, 1996). Then there is a further switch to 5-merous whorls by an additional double position. The innermost whorl of stamens may be incomplete on one side because of the asymmetric shape of the floral apex.

These switches in merosity do not always take place at the same site. All eight flowers studied begin with three 2-merous alternating whorls of tepals, followed by a 4-merous whorl of tepals (by double positions on the broader sides). In most flowers, the next whorl of tepals is 5-merous. Then two 5-merous whorls of stamens follow. The third whorl of stamens is either also 5-merous or there are only three (Fig. 2) or two stamens on one side of the flower (because the floral apex is slightly asymmetric). The gynoecium is 2-merous. In two of the flowers studied the transition to 5-mery was only in the second whorl of the androecium. One flower had two and one had three 4-merous whorls of tepals, and in both of them the first whorl of stamens was also 4-merous.

Thus the number of floral organs in the eight floral buds studied was: tepals (14–)15(–18), stamens 12–16, carpels 2. The previous counts by Capuron (1963), Baranova and Leroy (in Leroy, 1978), and Vink (1978) are all in this range, except that Vink found only 12 perianth parts.

PERIANTH

Winteraceae commonly have 2 (rarely 3) outer perianth organs that are more or less congenitally united and form a tight cover over the floral bud, which is often called the involucre or calyptra (e.g., Vink, 1988). The other perianth organs are free or more rarely some outer ones are (always postgenitally?) united. Terminology of the perianth organs of Winteraceae is not uniform in the previous literature. The involucre or calyptra was variously interpreted to be made up of bracts or sepals. The other perianth organs were variously called tepals, sepals, or petals (e.g., Nast, 1944; Capuron, 1963; Baranova & Leroy in Leroy, 1978; Vink, 1978; Gottsberger et al., 1980). There is no clear distinction between sepals and petals either in Winteraceae or in other basal angiosperms. Therefore, we prefer to use the term tepals for all perianth organs including those of the involucre (Hiepko, 1965; Endress, 1996).

In *Takhtajania,* the outer two tepals are congenitally united to form an involucre. Since they are much shorter than the other tepals, they do not pro-

tect the inner organs in older buds. Already in bud they form a more or less horizontal platform, which is somewhat elongated in the plane of its two tepals. The next inner four tepals (two pairs) form the topographical periphery of old floral buds. The outer two slightly overlap the inner two. In the overlapping region they are postgenitally united for about half of their length by interdentation of the cuticle (Figs. 2, 4A, B). As the flower opens this bond ruptures, and these four tepals are the first to spread. On their outer surface these four tepals are smooth and have a thick cuticle; on their inner surface they are papillate. All the following tepals have papillate surfaces. This papillate epidermis is tanniferous, as are the one or two cell layers below the epidermis. The margins of these tepals are not tanniferous; the cells are less vacuolate and have relatively large nuclei (Fig. 4C). From their appearance they seem to be secretory, and in microtome sections blue-staining secreted material is often concentrated around the tepal margins. This secretion was also observed in the field in June 1998 by G. Schatz. In the colored photographs of an open flower in Schatz et al. (1998) and in Schatz (2000 this issue), the red petals have white margins that correspond to this secretory zone. The material investigated contained insect (thrips?) larvae in the flower buds. It is uncertain whether these insects caused some of the secretion. Intercellular spaces in the tepals are not extensive. Starch is present especially around the vascular bundles.

The inner tepals commonly have three vascular bundles at their base, whereas the outer tepals have more. However, there is only one vascular strand from each tepal joining the stele in the floral base.

ANDROECIUM

At anthesis the stamens have apical extrorse anthers on club-shaped filaments (broadest shortly below the anther). Thus the thecae are basally spreading and almost horizontal. At the transition from the female to the male phase of anthesis, the filaments elongate and considerably thicken in the upper part, while the anthers shrink as they open (Fig. 5). As a result, the proportions of the stamens change considerably during anthesis. The filaments are more or less circular or slightly broader than thick in transverse section. The epidermis is slightly papillate and more or less tanniferous. Tanniferous tissue also occurs in scattered patches below the epidermis but is largely lacking in the center of the filament. This tanniferous region extends up to the ventral surface of the anther connective. Oil cells are present. Cells with oxalate crystals are lacking. The stamens contain a single collateral vascular bundle. The tissue around the vascular bundle contains abundant starch before the filament expands. After expansion the starch has disappeared (Fig. 6). Expansion of the filament goes hand in hand with cell enlargement.

GYNOECIUM

The gynoecium is disymmetric and club-shaped (Fig. 7D, E). It consists of two congenitally united carpels (Leroy, 1977; Vink, 1978). In young floral buds the tips of the two carpels can clearly be distinguished (Fig. 8A, B). At anthesis each carpel has a longitudinal furrow on its dorsal side (Fig. 7A, D). There are stomata on the outer surface (Fig. 7F). The gynoecium has a single locule. In the terminology of Leinfellner (1950) the entire ovary is symplicate; there is no synascidiate part at the base. The inner surface of the gynoecium is secluded from the outside by a completely postgenitally fused slit, which at the surface extends as a line between the two carpels (Figs. 3A, 9B). In the middle this line is crossed by a more or less distinct transverse furrow (Fig. 7B). The entire slit is surrounded by the stigma, which forms a large convex cap atop the gynoecium. The stigmatic zone is more extended in the commissural region than in the median plane of the carpels (Fig. 7D, E). The broadest part of the gynoecium, the ovary, is slightly above mid-length. Above the ovary there is a massive part with the common pollen tube transmitting tract (Figs. 3A–D, 9A–E). Below the ovary is a relatively long solid base, the common stipe of the two carpels (Fig. 3I–K). Although the ovary is unilocular, the placentae of the two carpels are obliquely directed; they meet in the center of the locule (Fig. 7G). They form an arch, which is deepest in the middle (Fig. 9A).

Each carpel has a dorsal vascular bundle, which extends up to some distance above the locule, where it may branch (Figs. 3A, 7G). The branches end about halfway between the level of the placentae and the stigma. Each carpel also has two or more lateral bundles, which are sometimes separate from the floral base (Figs. 3D–K, 7G). They flank the placentae and serve the ovules. They are connected with the dorsal bundles outside of the placentae and may also show connections between each other (Figs. 3C, 9C). The dorsal bundles commonly have two xylem portions that are directed toward each other and two phloem portions directed away from each other, which gives the appearance of two bundles (Fig. 3D–K). However, they probably originate from a single bundle. The same dou-

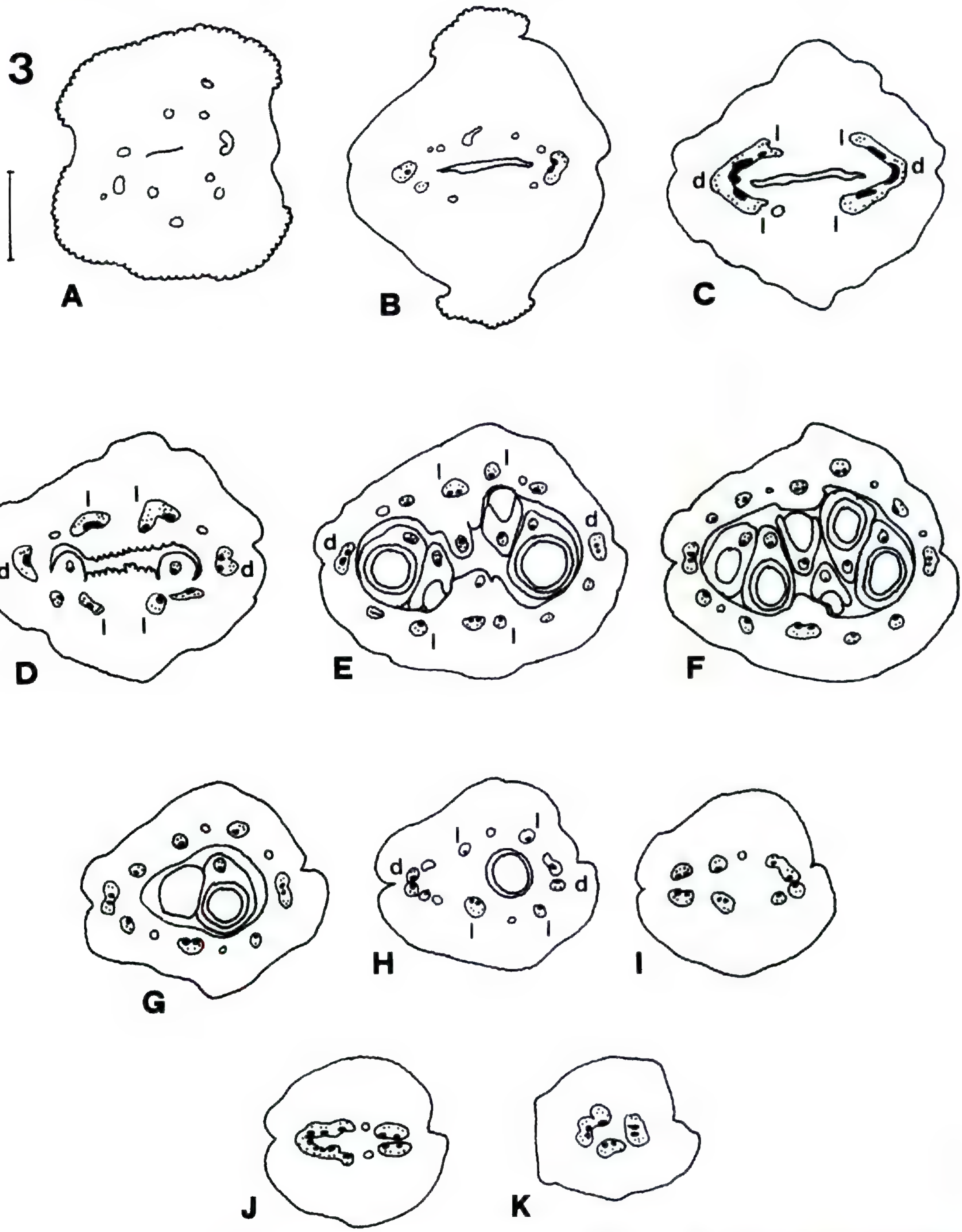


Figure 3. *Takhtajania perrieri.* Gynoecium at anthesis, transverse section series (d: dorsal vascular bundle, l: lateral vascular bundle). —A. Stigmatic zone, inner surfaces of gynoecium postgenitally fused. —B. Lowermost stigmatic zone, inner surfaces not fused. —C. Zone above ovary, lateral carpel vascular bundles merge with dorsal bundles. —D. Upper placental zone. —E. Lower placental zone. —F. Ovary with all ovules. —G, H. Ovary base. —I–K. Solid base of gynoecium with rearrangement of vascular bundles. Scale bar = 0.5 mm.

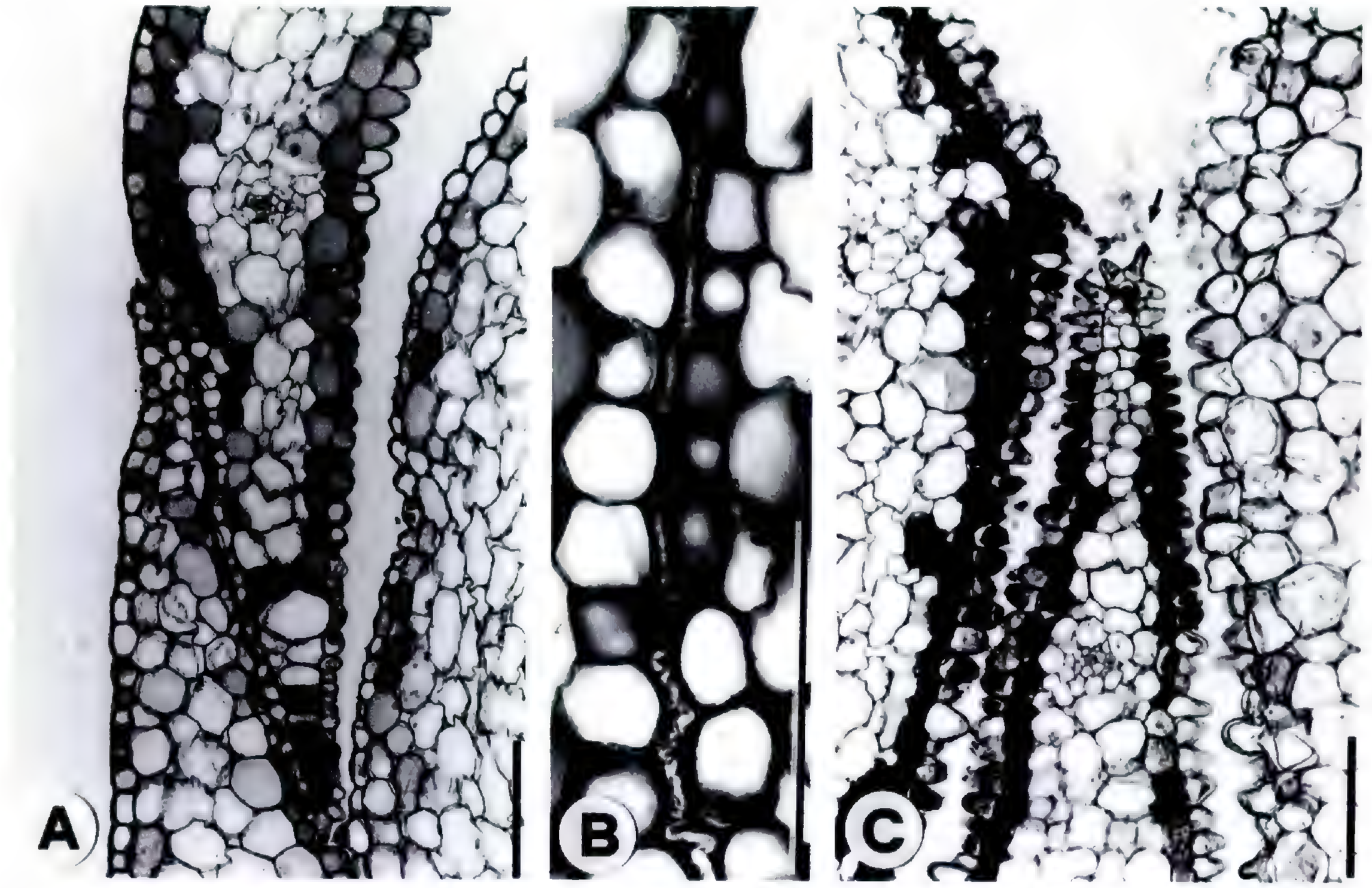


Figure 4. *Takhtajania perrieri.*—A. Transverse section of outer tepals in floral bud, showing postgenital fusion of overlapping area. —B. The same in higher magnification, showing interdigitation of cuticles of the postgenital fusion area. —C. Transverse section of non-tanniferous margin area of inner tepal, surrounded by secreted material (arrow). Scale bars in A and C = 0.1 mm, in B = 0.05 mm.

ble appearance also occurs in strong lateral bundles. Thus the ovules are served primarily by the lateral bundles, and not by the dorsal bundles (in contrast to the interpretation by Deroin & Leroy, 1993; see discussion) (Fig. 7G).

The stigmatic surface is unicellular-papillate and secretory (Fig. 7C). Also the pollen tube transmitting tissue that lines the inner gynoecium surface down to the placentae has large, unicellular papillae (Fig. 9E). The stigmatic papillae are tanniferous and the several cell layers of the tissue below them still more so (Fig. 9A, B). Below the region of the pollen tube transmitting tissue, the inner surface of the ovary is lined with about two layers of tanniferous cells. Throughout the gynoecium there are scattered tanniferous cell groups. The gynoecium wall also contains ethereal oil cells. Cells with oxalate crystals are present. The inner layers of the ovary wall contain abundant starch. Stomata are scattered over the surface of the gynoecium, especially in its upper part. Stone cells were not found in the gynoecium.

In 31 flowers studied we found (5–)6–7(–8) ovules per gynoecium (1 with 5, 9 with 6, 16 with 7, 5 with 8 ovules). Capuron (1963) and Leroy (1978) mentioned 5–11 ovules for the type material; however, in the figures by Vink (1978) and Deroin and Leroy (1993) there are only four ovules in a gynoecium. The ovules are pendent and are arranged in two lines. They are bitegmic, crassinucellar, and anatropous (Fig. 10). They are ca. 900 μm long. Ovule width/length ratio is 0.6. The nucellus is ca. 280 μm broad. The micropyle is formed by the inner integument (Fig. 10). The outer integument is semiannular, and the inner is annular (Fig. 10A, B). However, of the 31 gynoecia studied we found two in which one of the six ovules was orthotropous and both integuments were annular; the orthotropous ovule was smaller than the other ones and had a long funicle. In transverse section the ovules are wedge-shaped because they alternate on both sides of the double placenta and are wedged together (Figs. 3F, 9F). The outer integument is 4–5 cell layers thick, and the inner is 3 cell layers thick. Tanniferous tissue occurs mainly in the periphery of the ovule (outer integument and raphe, especially around the vascular bundle).

Discussion

Number and phyllotaxis of floral organs

Lability of floral organ number and phyllotaxis, and the tendency toward some irregularity and

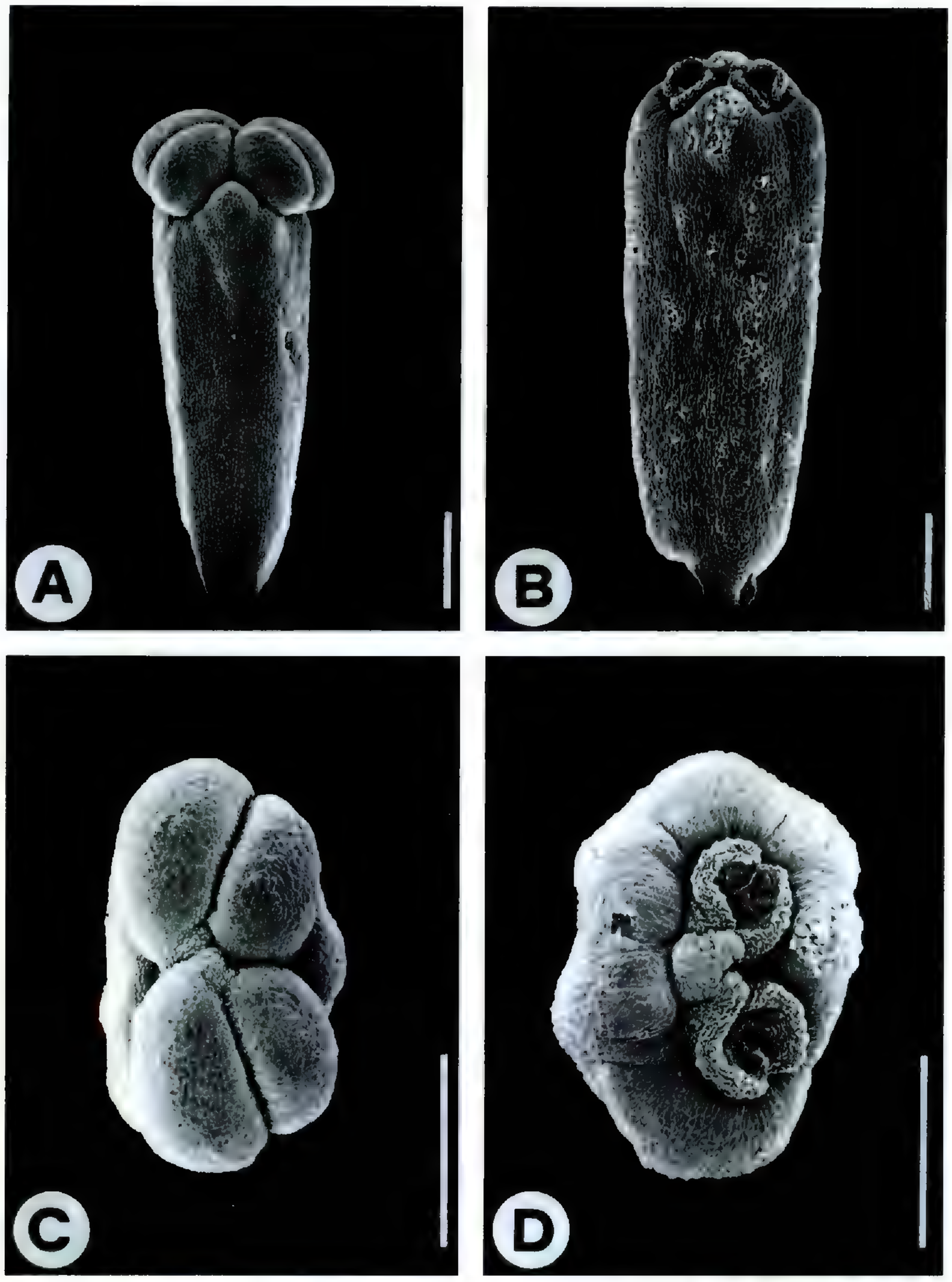


Figure 5. *Takhtajania perrieri.* Inner stamen in female and male phase of anthesis; in the male phase the filament is inflated and the anthers are open and shrunken; each pair of figures is at the same magnification. A, B. From dorsal side. —A. Female phase. —B. Male phase. C, D. From above. —C. Female phase. —D. Male phase. Scale bars = 0.5 mm.

asymmetry, is common in flowers of Winteraceae and is also present in *Takhtajania.* Floral phyllotaxis seems to be predominantly irregularly whorled in Winteraceae (Vink, 1970, 1977, 1978, 1985, 1993b; Endress, 1986, 1987). From the record in the literature, *Drimys winteri* seems to be an interesting exception with more or less regular spiral floral phyllotaxis (Hiepko, 1966; Erbar & Leins, 1983). However, Ronse Decraene and Smets (1998) mentioned chaotic floral phyllotaxis also for *Drimys winteri.* Doust (1997) shed light on this seeming contradiction by his observation that terminal flowers in *Drimys winteri* have a more or less spiral pattern, while lateral flowers have more chaotic patterns due to initial asymmetries of the floral apex (see also Vink, 1970); Doust (1997) also found that terminal flowers have more floral organs than lateral ones.

Notwithstanding this irregularity, there is a most common pattern within the family: The outer floral organs are decussate. Then there is a change to 4-merous alternating whorls and sometimes, by additional double positions, to whorls with a higher number of organs. This was reported to be common for *Pseudowintera* (less common in *Tasmannia*) (Vink, 1970), and for *Zygogynum* (Vink, 1977, 1983, 1988). As shown here, it is also present in *Takhtajania.*

PERIANTH

In *Takhtajania* the two outermost, congenitally united tepals, which form the involucre, are much

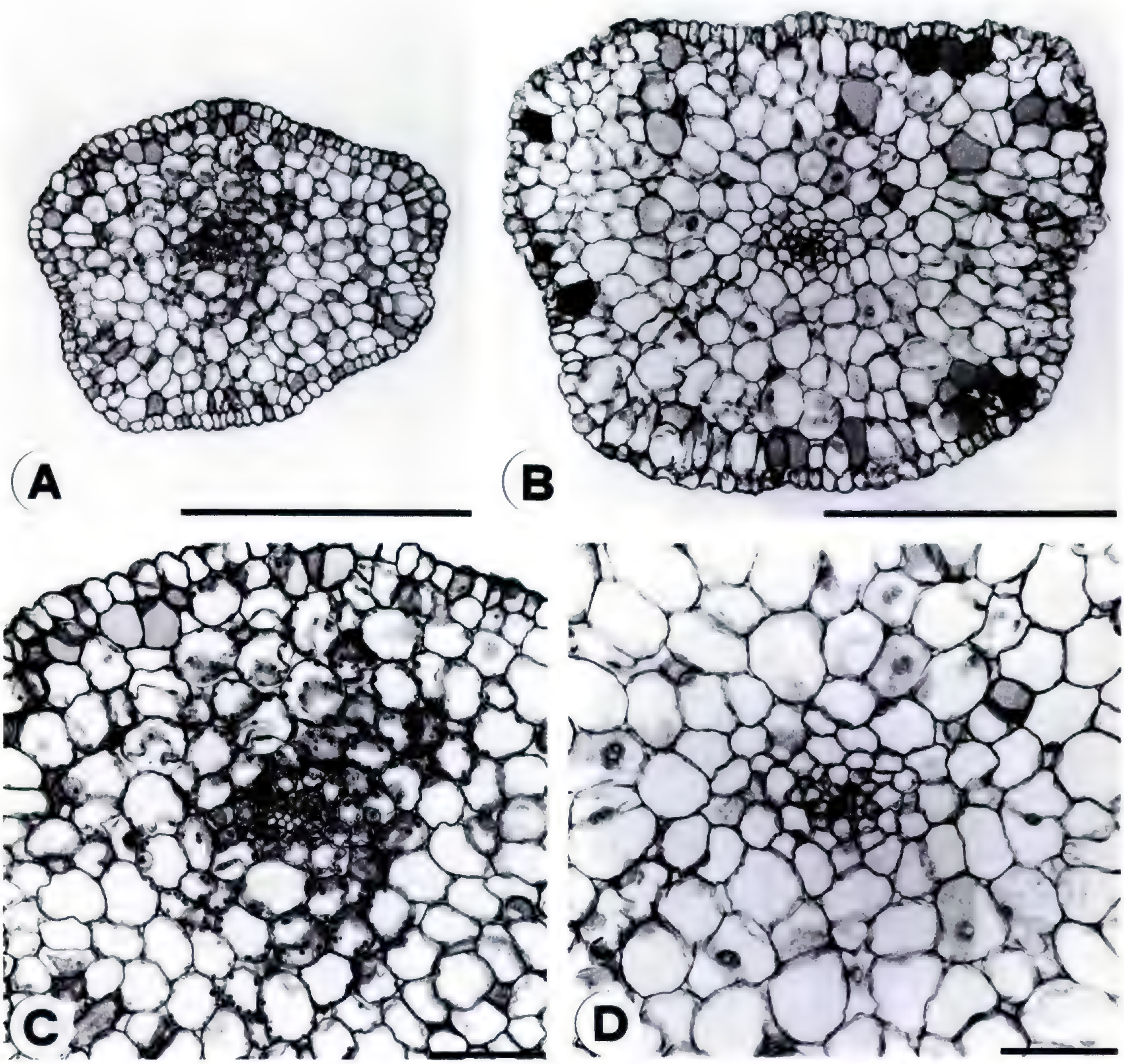
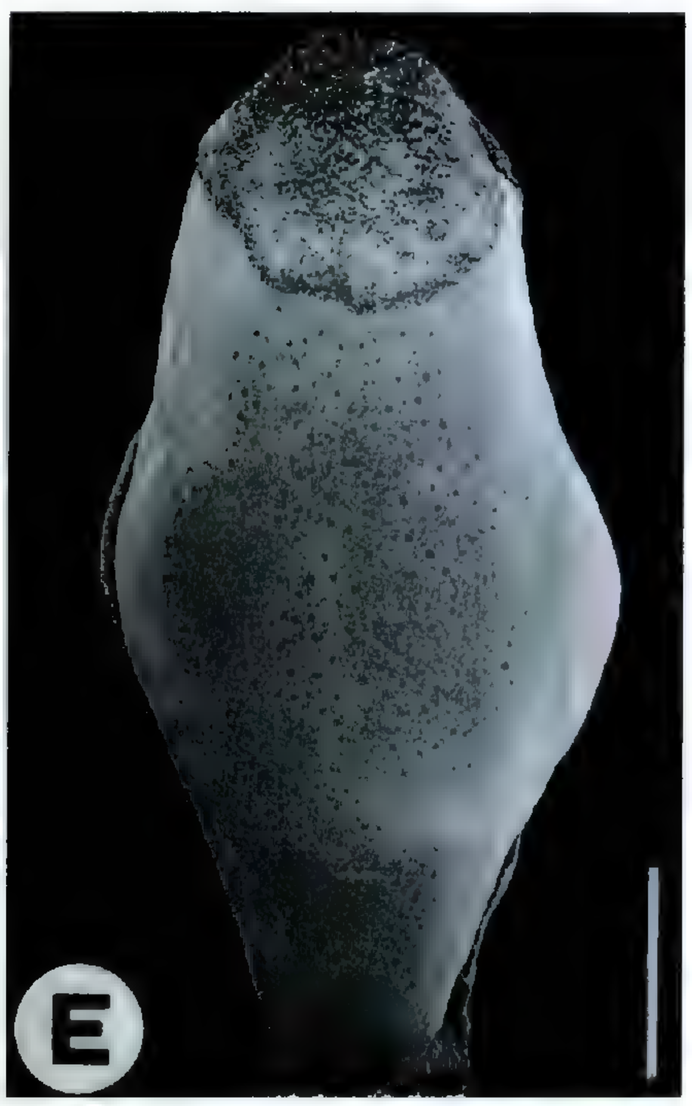
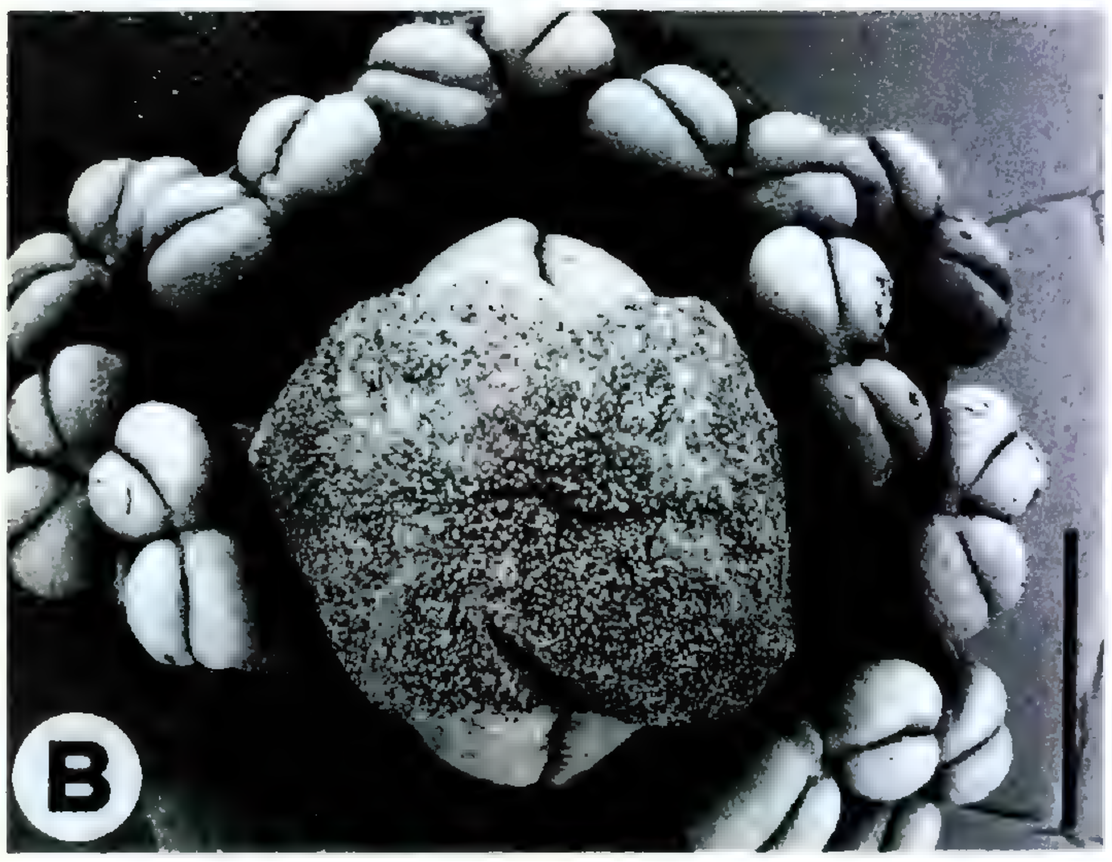
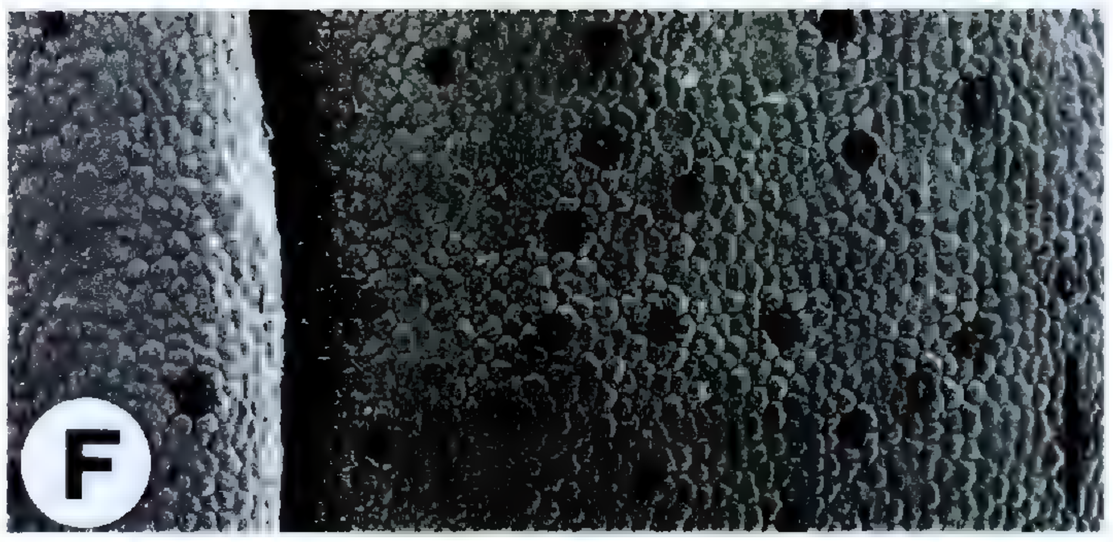


Figure 6. *Takhtajania perrieri.* Transverse sections of filaments of inner stamens immediately below anthers, in female and male phase of anthesis; in the male phase the filament is inflated by cell enlargement, and starch, which was present in the female phase, has disappeared; each pair of figures is at the same magnification. A, B. Entire transverse section. —A. Female phase. —B. Male phase. C, D. Central part of A and B at higher magnification. —C. Female phase (note starch grains in tissue around vascular bundle: dark dots). —D. Male phase (starch grains no longer present). Scale bars in A and B = 0.5 mm, in C and D = 0.1 mm.

shorter than in *Drimys* and *Tasmannia,* where they are protective organs for the buds. In *Drimys* and *Tasmannia* the involucre encloses the other floral parts until the flower opens, while in *Zygogynum* and *Pseudowintera* it ruptures very early and the next inner tepals attain a protective function (Vink, 1988). The next inner whorl of tepals is postgenitally united in *Takhtajania.* Such union is also reported for some *Zygogynum* species but not for other Winteraceae (Vink, 1985, 1988; although without indication whether it is postgenital or congenital). We did not find tepals with secretory margins in *Drimys* and *Tasmannia.* In addition, the epidermis was tanniferous and not papillate in those genera. Tepals are white in *Drimys* and *Tasmannia* because of large intercellular spaces in the mesophyll, which form an optical tapetum that reflects incoming light. *Takhtajania,* in contrast, without an optical tapetum, has red tepals, as do some species of *Zygogynum,* but those have much thicker tepals (see Thien, 1980; Vink, 1993a). In addition to *Takhtajania,* some other Winteraceae contain starch in the tepals, which, at least in some, may provide food for pollinators (beetles, thrips) (Pellmyr et al., 1990; Thien et al., 1990).







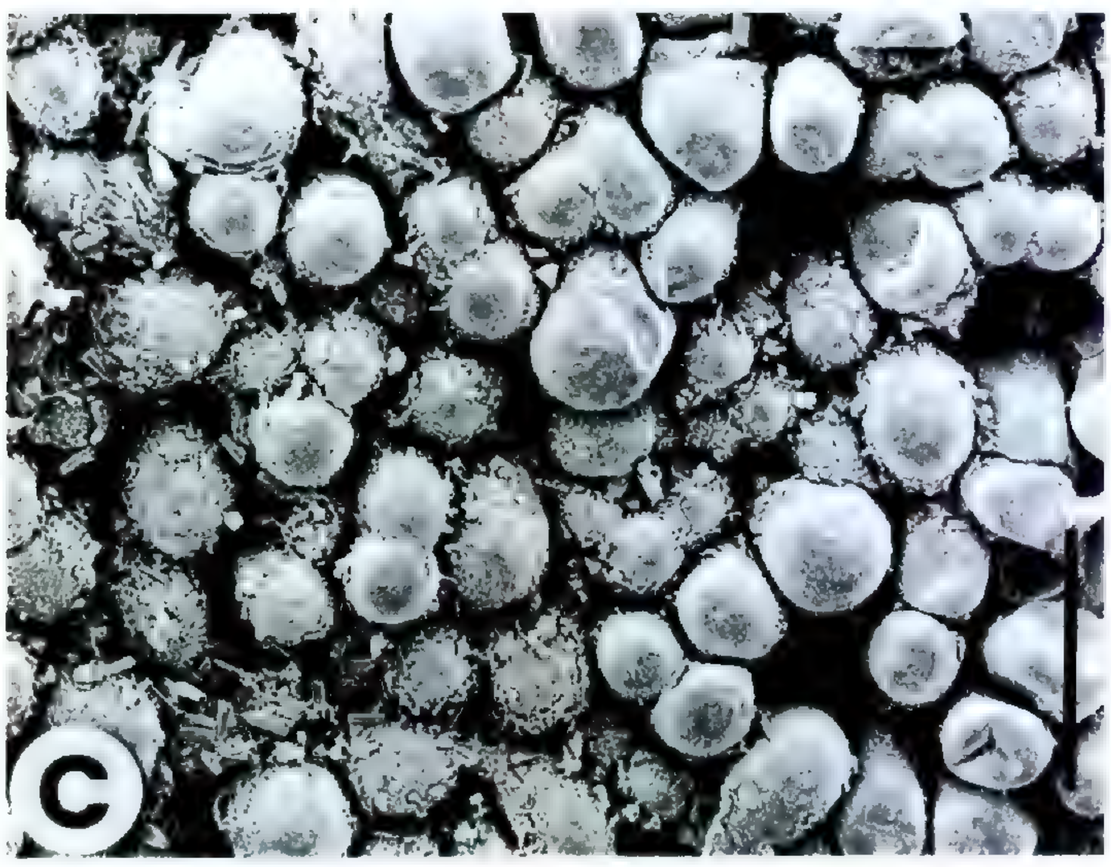


Figure 7. *Takhtajania perrieri.* A–C. Flower in female phase of anthesis. —A. Flower from the side, tepals broken off. —B. From above. —C. Stigmatic papillae with secretion. D–F. Gynoecium, from the side, shortly after anthesis. —D. Showing dorsal side of one of the two carpels, with longitudinal furrow. —E. Gynoecium rotated at 90°. —F. Magnification of D, showing stomata on gynoecium surface. —G. Confocal laser photograph showing vasculature (xylem) of a gynoecium (same view as E), arrows = dorsal vascular bundles, arrow-heads = lateral vascular bundles. Scale bars in A, B, D, E, and G = 1 mm, in C = 0.05 mm, and in F = 0.1 mm.

Figure 8. *Takhtajania perrieri.* Gynoecia of young floral buds, from the side. —A. Before beginning of stigma formation. —B. At beginning of stigma formation. Scale bars = 0.1 mm.

ANDROECIUM

Club-shaped filaments with the thecae on top as in *Takhtajania* also occur in *Pseudowintera* and *Zygogynum.* In *Drimys* and even more so in *Tasmannia* the filaments are thinner and the thecae are less terminal and more lateral (Bailey & Nast, 1943a; Sampson, 1987; Endress & Hufford, 1989; Endress, 1994).

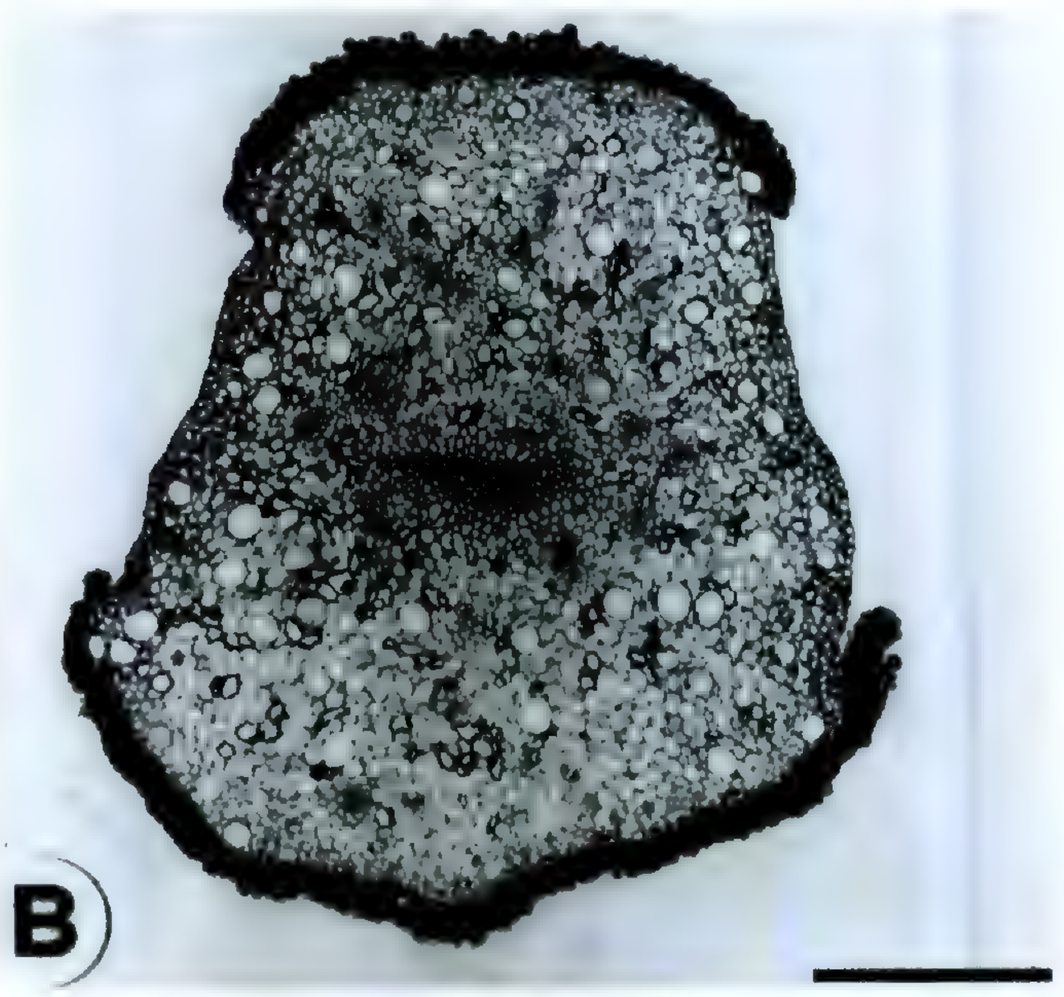
The behavior of the stamens of *Takhtajania* during anthesis with elongation and especially thickening and broadening of the filament is also characteristic for other Winteraceae. It was shown for *Pseudowintera* by Sampson (1980) and Lloyd and Wells (1992), and for *Zygogynum mackeei* Vink, *Z. stipitatum,* and *Z. pancheri* by Carlquist (1981, 1982, 1983). Carlquist (1982) also noted the decrease in starch content in stamens of *Z. stipitatum.* He discussed filament expansion in the context of flower opening by pressure of the stamens. However, we found the conspicuous filament expansion only after flower opening, at the transition from the female to the male phase and interpret it as associated with pollen presentation (see also Sampson, 1980, for *Pseudowintera*). Loss of starch may be correlated with rapid cell growth in this phase, as also indicated by Carlquist (1982) for *Zygogynum stipitatum.* It should also be studied whether starch loss is here associated with scent production, as this often occurs in osmophores (Vogel 1990). Pellmyr et al. (1990) discussed the significance of floral scents in Winteraceae for pollination but did not mention the source of the scents (see also section on perianth).

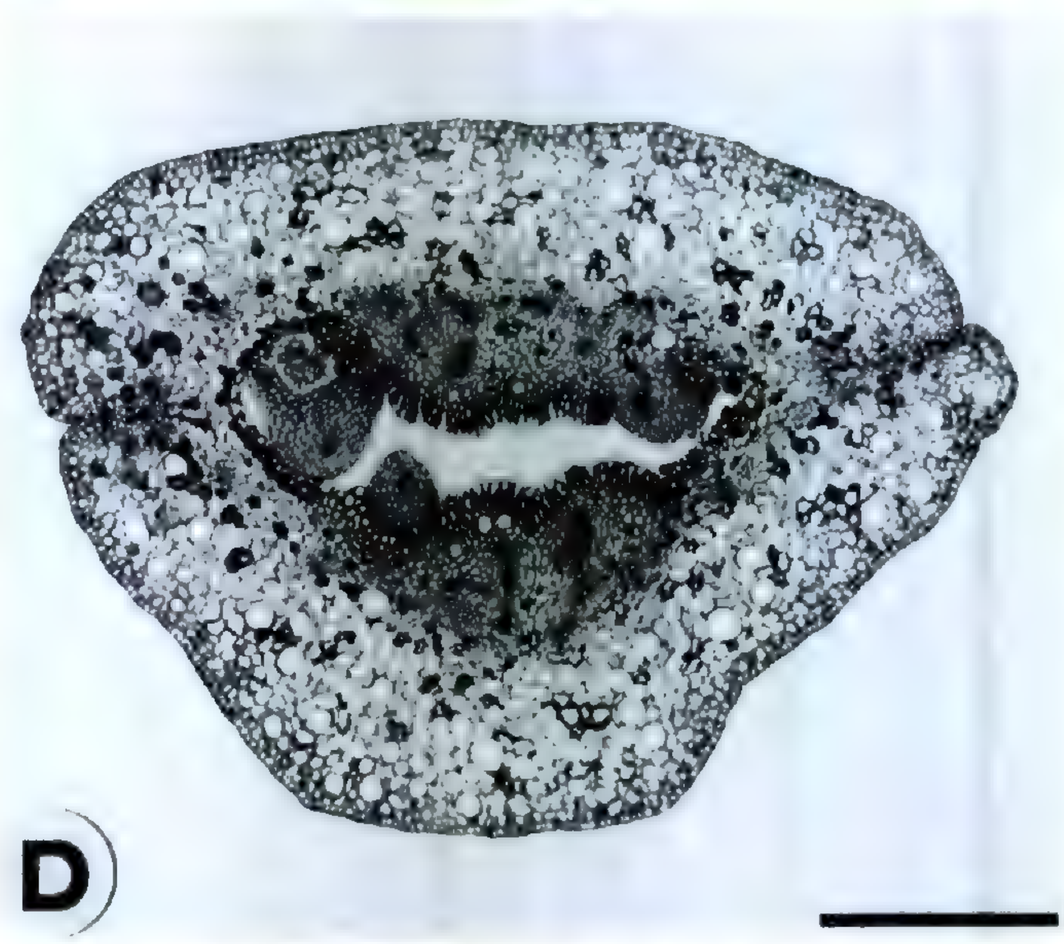
Since the thecae in *Takhtajania* are on top of the club-shaped filaments, the position of the thecae is highly oblique to almost horizontal (also in *Pseudowintera* and *Zygogynum*). Therefore, in transverse sections of stamens the thecae are cut obliquely. As a consequence, the endothecium, which is one-layered, may appear to be two- or more-layered (see Swamy, 1952, for *Zygogynum baillonii*), while in reality it is only one-layered.

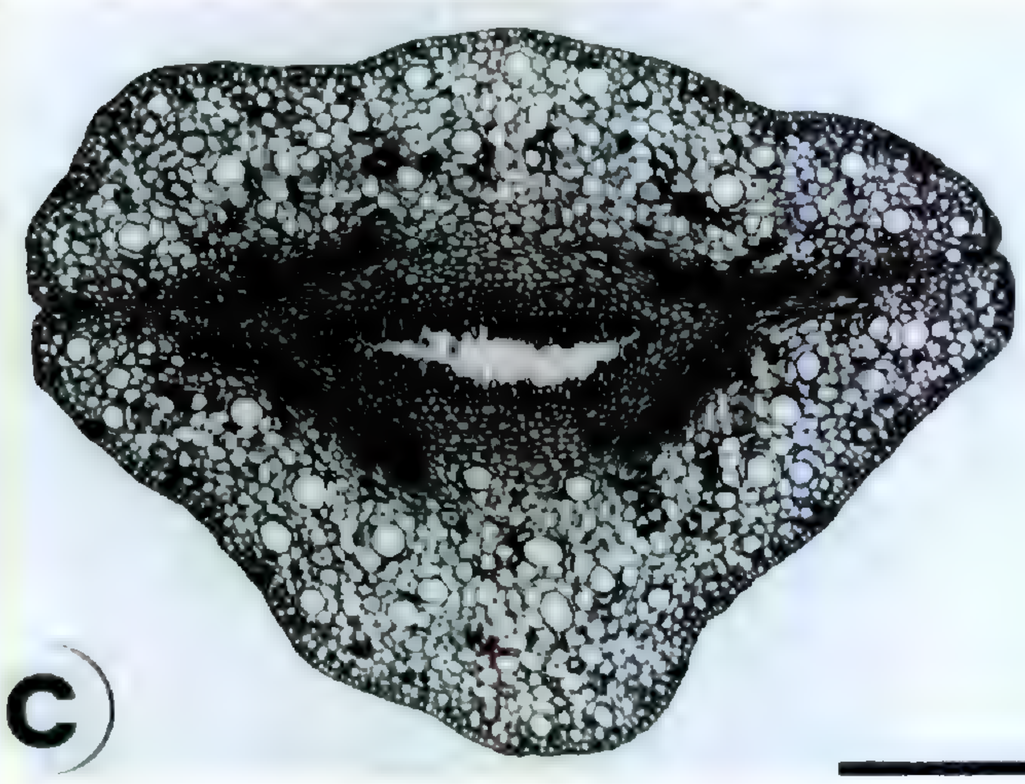
GYNOECIUM

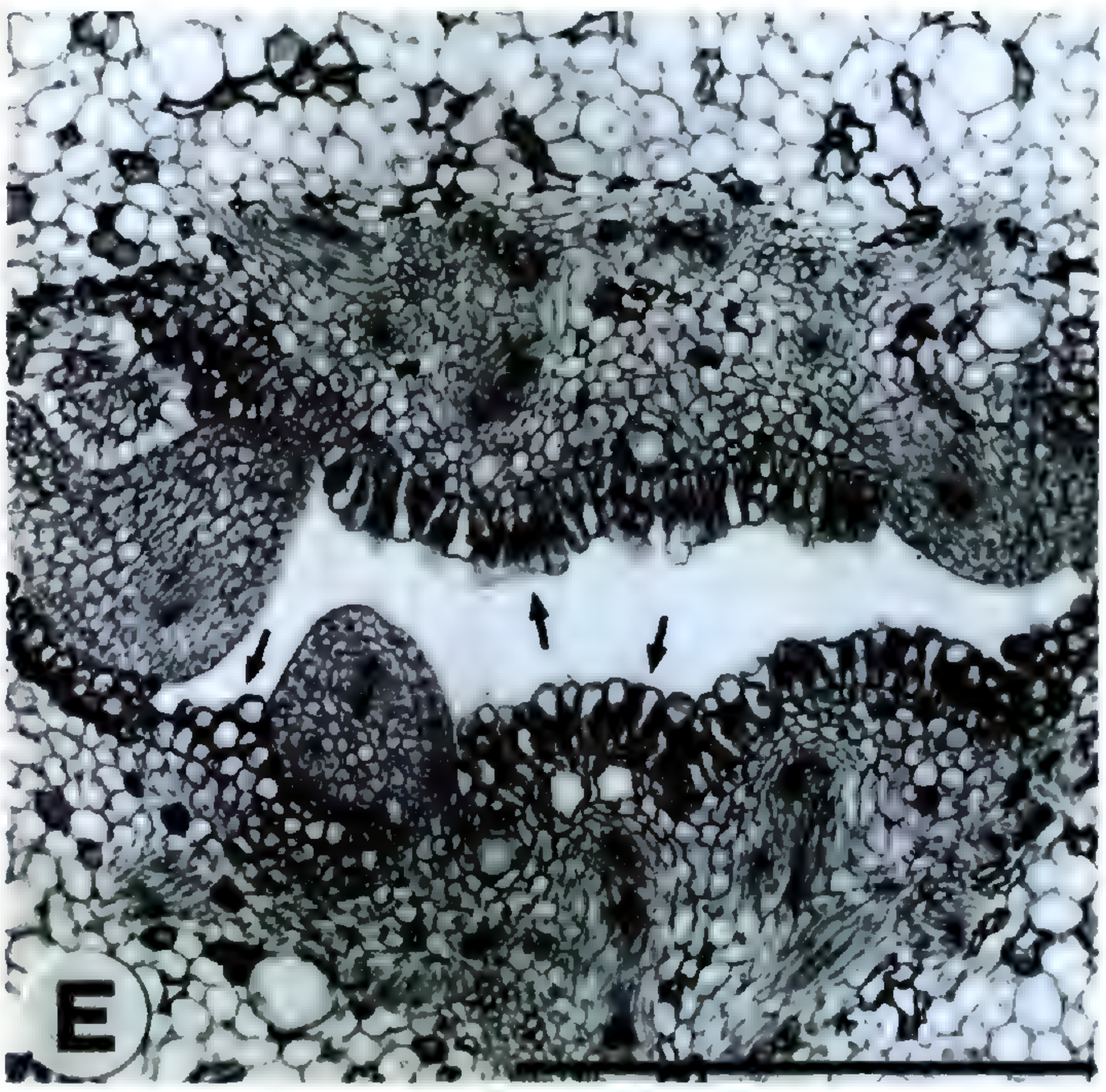
The gynoecium of *Takhtajania* is peculiar. Although it is bicarpellate and unilocular, it is not paracarpous in the normal sense with parietal placentation. The placentae are not vertical but oblique to almost horizontal. Therefore, the placentae of both carpels are separate and are only contiguous at their morphological bases. Thus, placentation is not laminar (as opposed to Leroy, 1993) but has a normal linear configuration. The stigma is topographically apical, but morphologically it surrounds the entire (postgenitally fused) entrance into the internal space of the two carpels. The stigma is not commissural either (as opposed to Leroy, 1980, 1993), because the entire orifice is stigmatic and not only the lateral parts, although the stigmatic surface is more extended in the commissural than in the median region. Thus, it corresponds to the stigma extension of most other Winteraceae, which have a double-crested stigma with the crests confluent at both ends.

This unique bicarpellate unilocular gynoecium of *Takhtajania* could have evolved from a unicarpellate ancestor. Unicarpellate gynoecia are known from species of *Tasmannia, Pseudowintera,* and *Zygogynum* (*Bubbia*) (Sampson, 1963; Vink, 1970, 1983, 1993a; Ueda, 1986). In these species, sometimes two carpels instead of one carpel develop in a flower (Sampson & Kaplan, 1970). In such gynoecia, the available space for two carpels is limited so that they may form a unilocular paracarpous structure, as Sampson and Kaplan (1970) showed for *Pseudowintera traversii* Dandy. Furthermore, in









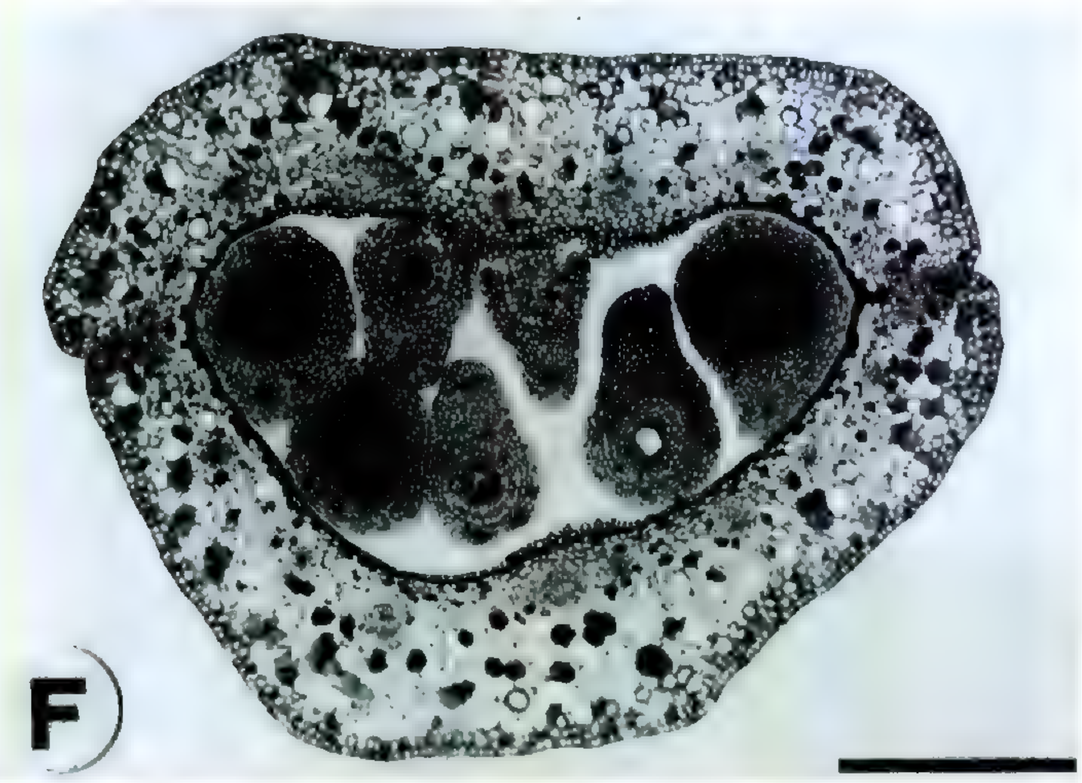


Figure 9. *Takhtajania perrieri.* —A. Gynoecium at anthesis, in approximately median longitudinal section. B–F. Gynoecium at anthesis, transverse section series. —B. Stigmatic zone, inner surfaces of gynoecium postgenitally fused. —C. Zone above placenta, inner surface not fused. —D. Upper zone of placenta. —E. Same in higher magnification, showing the papillate pollen tube transmitting tissue (arrows). —F. Ovary, showing the transversely sectioned wedge-shaped ovules. Scale bars = 0.5 mm.

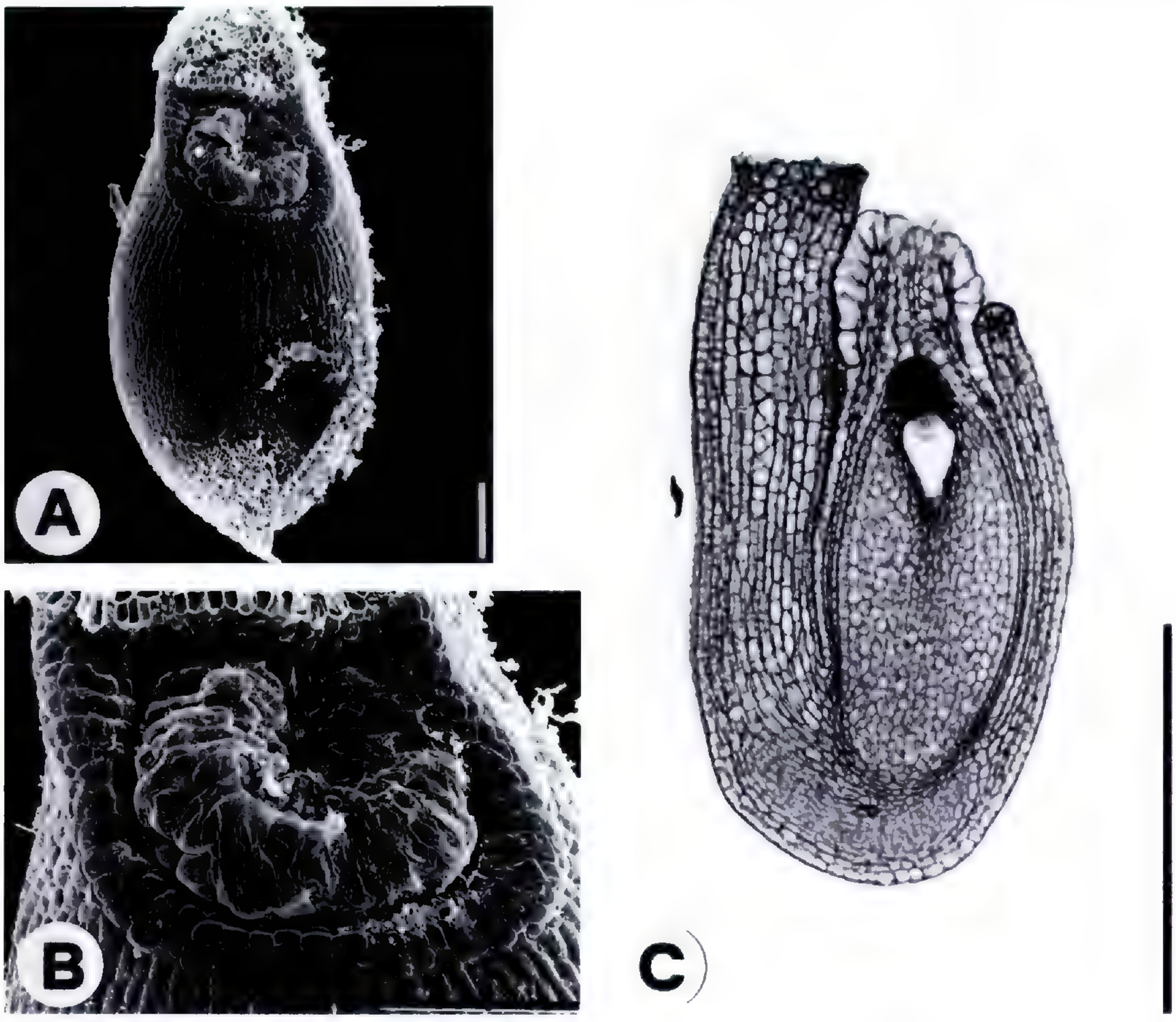


Figure 10. *Takhtajania perrieri.* Ovules at anthesis. —A. Ovule from micropylar side, micropyle formed by the inner integument (arrow), outer integument semiannular. —B. Same, micropylar part in higher magnification. —C. Ovule in approximately median longitudinal section. Scale bars in A and B = 0.1 mm, in C = 0.5 mm.

Zygogynum two (or three?) central stigmas of a gynoecium may be confluent (*Z. baillonii,* Vink, 1993a). A dorsal furrow in the carpels as in *Takhtajania* also occurs in *Tasmannia lanceolata* (Leinfellner, 1965; Vink, 1970; Leroy, 1980). The position of the furrows is dorsal because they alternate with the two placentae in the bicarpellate, syncarpous gynoecium of *Takhtajania.* In the free carpels of *Tasmannia,* the furrow lies opposite the placenta. The significance of the furrows is not clear; dehiscence of the mature fruits has not been reported. Another, more conventional hypothesis is evolution of the gynoecium of *Takhtajania* from two free carpels.

In their discussion, Deroin and Leroy (1993) mentioned the apical placenta of *Takhtajania* as peculiar. This differs from the gynoecium in paracarpous Annonaceae, with which they made a comparison. However, it is not peculiar within Winteraceae, because the majority of them have "apical" placentae (because of their more or less horizontal direction).

How does the gynoecium of *Takhtajania* compare with that in other Winteraceae (apart from its peculiar syncarpy)? Several authors published studies on more than one genus of the Winteraceae that may serve as a comparative basis for this question: (1) the studies by Bailey and Nast (1943b, 1945) and Bailey and Swamy (1951) especially focused on the vasculature; (2) the studies by Tucker (1959, 1975), Tucker and Gifford (1964, 1966a, b), Sampson (1963), Sampson and Kaplan (1970), and Sampson and Tucker (1978) concentrated on the morphological and anatomical development of carpels, vascularization, and placentation; (3) the studies by Leinfellner (1965, 1966a, b, 1969) primarily dealt with the outer and inner morphology and placentation; (4) the study by Igersheim and Endress (1997) focused on morphology and histology of carpels and ovules in comparison with that in other Magnoliales and winteroids.

Leinfellner (1965, 1966a, b, 1969) and Tucker and Gifford (1966b) found an unusually high variability of carpel shapes in Winteraceae from highly ascidiate to largely plicate. Non-ascidiate carpels as in *Takhtajania* are only known from *Tasmannia* (see also Frame, 1996). However, these are not directly comparable, because the non-ascidiate shape in *Takhtajania* may be caused by its syncarpy, while *Tasmannia* is apocarpous.

Gynoecium vasculature of *Takhtajania* is not different from that in other Winteraceae. The ovules are served primarily by lateral bundles (see also Vink, 1978), and not predominantly by the dorsal bundles as contended by Deroin and Leroy (1993). Carpels in Winteraceae generally have a dorsal vascular bundle, which has sometimes been characterized as "double," or there are two dorsal bundles, such as in *Tasmannia* (Tucker & Gifford, 1964); in addition, there are two ventral (lateral) bundles associated with the placentae, which may merge into one bundle in the ascidiate basal part of the carpel. Dorsal and lateral vascular bundles may be connected by secondary bundles later in development. In a critical study Tucker (1975) showed that ovules are principally served by lateral carpellary vascular bundles in species of *Drimys* and *Tasmannia*. In contrast, Bailey and Nast (1943b) had described the ovules as being vascularized partly by branches of the dorsal strands, and partly by anastomoses between dorsal and ventral strands. It seems to be a peculiarity that the ovular vascular strands differentiate relatively late, when the dorsal and ventral vascular bundles are already far differentiated. This is probably due to the fact that the ovules arise relatively late, when the carpels are already relatively massive and the primary vasculature is relatively advanced in development. As a consequence, the ovular traces connect with secondary vascular bundles between the lateral and dorsal main strands that have formed later. However, the connection with the lateral vascular bundles is still there (Tucker, 1975; see also Ueda, 1978). Likewise, in *Takhtajania*, the ovules are served by lateral vascular bundles or by connections between the dorsal and lateral ones (and not by dorsal ones as Deroin & Leroy, 1993, described).

The ovules of *Takhtajania* are much larger at anthesis than those in all other taxa of Winteraceae studied (see list in section "Material and Methods"). The ovules of *Takhtajania* are 900 μm long, whereas those of the other taxa investigated vary between 330 μm in *Tasmannia insipida* and 625 μm in *Zygogynum baillonii*. This may be correlated with the low number of ovules per ovary in *Takhtajania* and the different architecture of the bicarpellate unilocular ovary as compared to the ovary in free carpels. In morphology and histology (especially distribution of tannins), the ovules are similar to those of other Winteraceae (see Strasburger, 1905; Bhandari, 1963; Sampson, 1963; Bhandari & Venkataraman, 1968; de Boer & Bouman, 1974; Prakash et al., 1992; Imaichi et al., 1995; Igersheim & Endress, 1997; Svoma, 1998).

Systematics of Winteraceae and Systematic Position of *Takhtajania*

Before recognition of *Takhtajania*, *Tasmannia* and *Drimys* were considered to be the basal branches in Winteraceae. *Tasmannia* was favored as the basalmost clade because of its low chromosome numbers (Ehrendorfer et al., 1968) and the conduplicate carpels (Smith, 1969) long viewed as a model for an archaic carpel form (Bailey & Swamy, 1951). *Drimys* was considered as the closest neighbor of *Tasmannia* because of many morphological similarities. In fact, for some time *Tasmannia* was subsumed under *Drimys*. However, chromosome studies by Ehrendorfer et al. (1968) prompted Smith (1969) to reinstate *Tasmannia*. The ITS studies by Suh et al. (1993) supported the split between the two genera. They also supported *Tasmannia* as sister of the rest of the family, which has *Drimys* in the basal position, followed by *Pseudowintera* and *Zygogynum* (the latter including *Bubbia*, *Belliolum*, and *Exospermum*, as proposed by Vink (1985) on morphological grounds; see also Vink, 1993b). Kubitzki and Reznik (1967) found a persistent difference in leaf flavonoids between *Drimys* and *Tasmannia*. The isolated position of *Tasmannia*, as well as the unity of the group *Bubbia*, *Belliolum*, *Exospermum*, and *Zygogynum*, was emphasized by Williams and Harvey (1982) based on the leaf flavonoid patterns. However, they interpreted *Tasmannia* as the most advanced genus in the family. On the basis of leaf epidermis, Baranova (1972) considered the basal dichotomy to be between *Drimys/Tasmannia* and *Bubbia/Belliolum/Pseudowintera/Zygogynum*; further, she emphasized the isolated position of *Bubbia perrieri*.

After *Takhtajania* was recognized as a separate genus, Vink (1988) explicitly proposed a basal position in the family for it, followed by a *Tasmannia/Drimys* clade. A basal position of *Takhtajania* had also been implied by Leroy (1978) by the erection of a subfamily Takhtajanioideae and later even a separate family Takhtajaniaceae (Leroy, 1980). Family status was later not accepted by other authors and was also rejected by Leroy (1993). Even before *Takhtajania* was erected as a genus and was still included in *Bubbia* as *B. perrieri*, Bongers (1973) found that alveolar material was present on the leaf surface of Winteraceae except for *Tasmannia* and *Bubbia perrieri*. In contrast, in view of its very large pollen tetrads and its particular pollen structure (Lobreau-Callen, 1977), which may indicate polyploidy, *Bubbia perrieri* was considered to be related to *Belliolum* (*Zygogynum*) and *Drimys* rather than *Tasmannia* (for correlation of chromo-

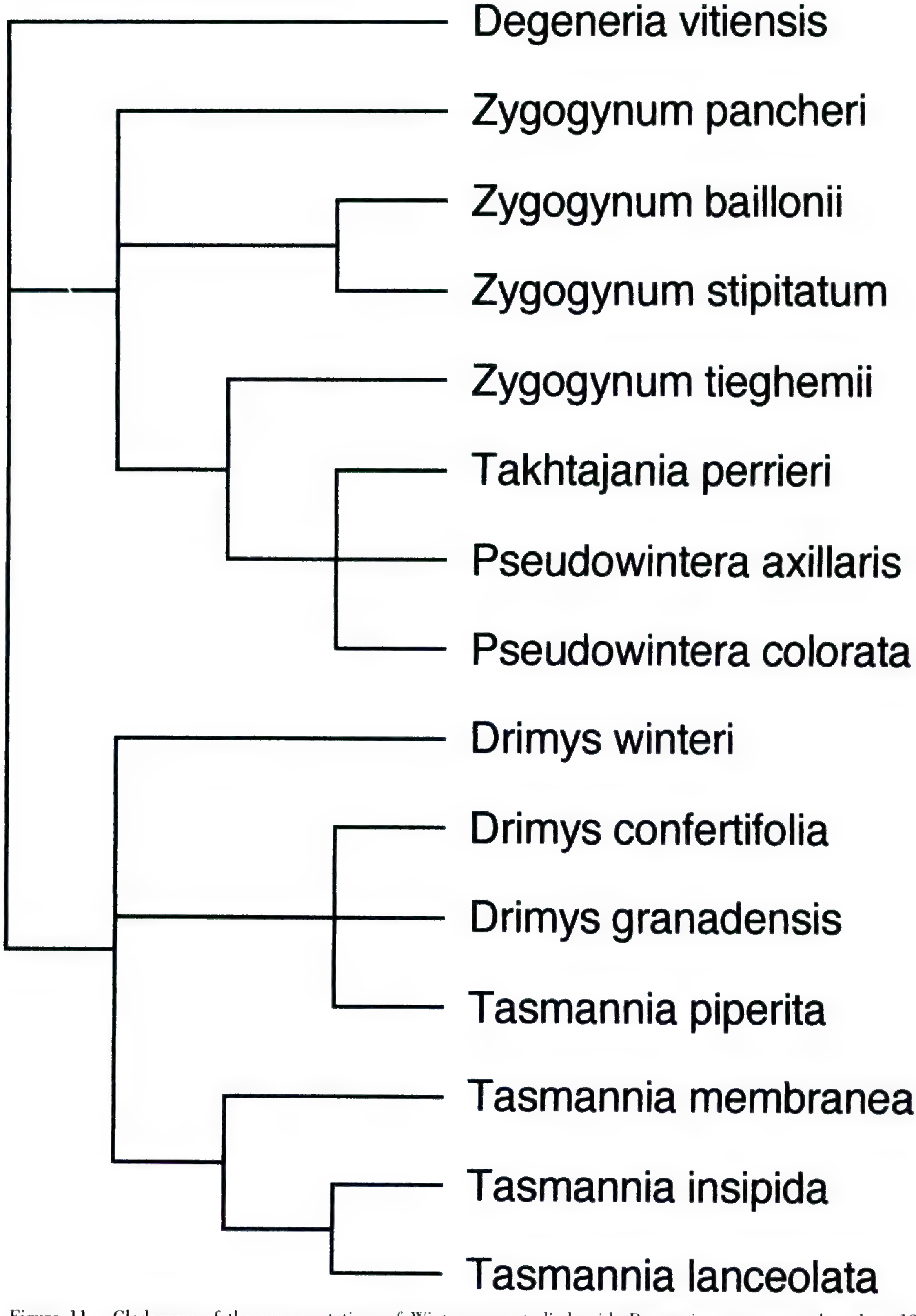


Figure 11. Cladogram of the representatives of Winteraceae studied, with *Degeneria* as outgroup, based on 13 representative floral features, showing *Takhtajania* nested in the *Pseudowintera/Zygogynum* clade (PAUP 3.1.1, heuristic search, TBR: consensus tree of 3 shortest trees with CI 0.765, RI 0.894, gained by reweighting with rescaled CI of 25 shortest trees of length 29 with CI 0.621 and RI 0.788).

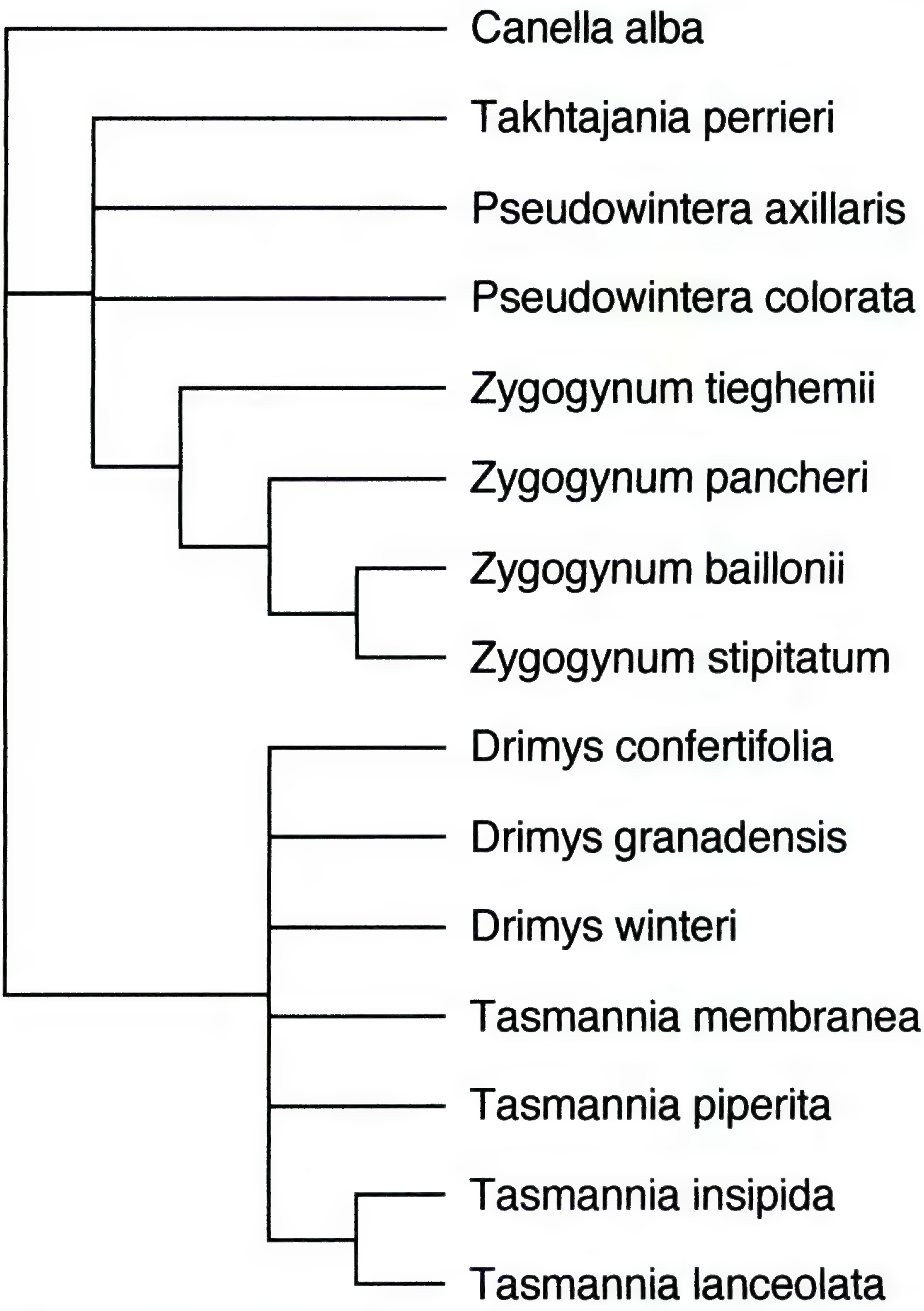


Figure 12. Cladogram of the representatives of Winteraceae studied, with *Canella* as outgroup, based on 13 representative floral features, showing *Takhtajania* nested in the *Pseudowintera/Zygogynum* clade (PAUP 3.1.1, heuristic search, TBR: consensus tree of 20 shortest trees with CI 0.713, RI 0.868, gained by reweighting with rescaled CI of 70 shortest trees of length 30 with CI 0.600 and RI 0.769).

some number and pollen size in Winteraceae, see Hotchkiss, 1955). Praglowski (1979) emphasized the special similarity of its pollen tetrads with those of *Drimys*.

From the results of the present comparative study of flowers of *Takhtajania* and other Winteraceae some new features come into the discussion. The particular club-shaped stamens and almost horizontal position of the thecae are shared with *Pseudowintera* and *Zygogynum*. The presence of an involucre that is short and protective only in young floral buds is shared with *Pseudowintera* and *Zygogynum*. The presence of whorls of 4 or 5 tepals is shared with *Pseudowintera* and *Zygogynum* species. The fusion of the tepals following the involucre is shared with some *Zygogynum* species. Red tepal color is shared with some *Zygogynum* species. This and the large pollen grains may indicate that *Takhtajania* constitutes a clade with the *Pseudowintera/Zygogynum* group and that a *Tasmannia/Drimys* group is sister to this clade (see also fig. 2.2 in Vink, 1988). This is also shown by a cladogram based on representative floral characters (Fig. 11; Appendixes 2, 3) and with *Degeneria* as an outgroup, a genus that tends to come out as sister group of Winteraceae in preliminary cladistic analyses based on gynoecium structures through all families of the basal angiosperms.

Another scenario, indicated by molecular data (Karol et al., 2000 this issue) shows a sister relationship of Winteraceae and Canellaceae. But even with Canellaceae as an outgroup, *Takhtajania* appears nested in a *Pseudowintera/Zygogynum* clade in the morphological analysis (Fig. 12; Appendixes 2, 3). If this scenario with Canellaceae sister to Winteraceae stands corroborated, the alternative view with *Takhtajania* basal in Winteraceae would be better supported. *Takhtajania* shares with Canellaceae having red flowers with whorled phyllotaxis and only a short involucre of united outer tepals. They also share a bicarpellate, paracarpous gynoecium, which, however, is different in detail. Canellaceae have vertical parietal placentae and campylotropous ovules with zig-zag micropyle, but *Takhtajania* has obliquely horizontal separate placentae that meet at their morphological base, and anatropous ovules with micropyle formed by the inner integument; see Igersheim & Endress (1997). Thus the paracarpous gynoecium is unlikely to be a synapomorphy for *Takhtajania* and Canellaceae. In addition to molecular studies, the karyotype of *Takhtajania* will be crucial in a phylogenetic interpretation.

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Drimys granadensis L.f. Costa Rica. Volcan Poas, *P. K. Endress 97-127.*

Drimys winteri J. R. & G. Forst. Switzerland. Cult. Isole di Brissago, *P. K. Endress 6524.*

Pseudowintera axillaris (J. R. & G. Forst.) Dandy. New Zealand, *P. K. Endress 6357.*

Pseudowintera colorata (Raoul) Dandy. Germany. Cult. Botanic Garden, University of Bonn, *P. K. Endress 4552.*

Takhtajania perrieri (Capuron) Baranova & J.-F. Leroy. Madagascar. Anjahanaribe-Sud RS., *P. J. Rakotomalaza et al. 1342, 13 VI 1997*; *G. E. Schatz 3748, VI 1998* (Fig. 1); *D. Ravelonarivo s.n., III 1998* (Fig. 8).

Tasmannia insipida R.Br. ex DC. Australia. Southern Queensland, *P. K. Endress 4294.*

Tasmannia lanceolata (Poir.) A. C. Sm. New Zealand. Cult. Botanical Garden, University of Canterbury, Christchurch, *D. G. Lloyd, s.n.*, 23 IX 1992.

Tasmannia membranea (F. Muell.) A. C. Sm. Australia. Northern Queensland, *P. K. Endress 4215.*

Tasmannia piperita (Hook. f.) A. C. Sm. Papua New Guinea, *P. K. Endress 4137.*

Zygogynum baillonii Tiegh. New Caledonia, *P. K. Endress 6295.*

Zygogynum pancheri (Baill.) Vink. New Caledonia, *P. K. Endress 6251.*

Zygogynum stipitatum Baill. New Caledonia, *A. M. Juncosa s.n.*, 1981.

Zygogynum tieghemii Vink. New Caledonia, *P. K. Endress 6329.*

Appendix 2. Matrix of 13 representative floral characters of *Takhtajania perrieri* and 13 other species out of all genera of Winteraceae.

	1	2	3	4	5	6	7	8	9	10	11	12	13
Takhtajania perrieri	1	0	1	1	1	1	1	1	0	0	1	1	1
Drimys confertifolia	2	1	0	0	0	1	0	1	0	1	0	0	0
Drimys granadensis	2	1	0	0	0	1	0	1	0	1	0	1	0
Drimys winteri	2	1	0	0	0	1	0	1	0	1	0	0	1
Pseudowintera axillaris	1	0	0	1	1	1	1	1	0	0	0	1	1
Pseudowintera colorata	1	0	0	1	1	1	1	0	0	0	0	1	1
Tasmannia insipida	0	1	0	0	0	0	1	0	0	1	0	0	1
Tasmannia lanceolata	0	1	0	0	0	0	1	0	0	1	0	0	1
Tasmannia membranea	0	1	0	0	0	?	1	1	0	1	0	0	1
Tasmannia piperita	0&1	1	0	0	0	0	0&1	1	0	0&1	0	1	0
Zygogynum baillonii	1	0	1	1	1	1	0	1	1	1	1	?	?
Zygogynum pancheri	1	0	0	1	1	0	0	1	1	1	0	1	1
Zygogynum stipitatum	1	0	1	1	1	1	0	1	1	1	0	1	0&1
Zygogynum tieghemii	1	0	0	1	1	?	1	1	1	0	0	0	1
Degeneria vitiensis	2	0	0	1	0	1	0	1	1	1	1	1	1
Canella alba	1	0	0	1	0	1	1	1	0	0	1	0	0

Appendix 3. Characters used for cladistic analysis.

1. Tepal number (involucre not counted) and arrangement: (0) 0–3, whorled; (1) 4 or more, whorled; (2) spiral.
2. Involucre (congenitally united outermost tepals) in advanced floral buds (Vink, 1993b): (0) shorter than other tepals; (1) as long as or longer than other tepals.
3. Postgenital (?) union of the tepals following the involucre (Vink, 1993a): (0) absent; (1) present.
4. Tepal color (Harden, 1990; Vink, 1993a): (0) white; (1) cream or red.
5. Theca position on the filament (Sampson, 1987): (0) ± vertical; (1) ± horizontal.
6. Pollen diameter (polar axis) (Praglowski, 1979): (0) 18 μm or less; (1) 19 μm or more.
7. Carpel number per flower (Vink, 1970, Sampson et al., 1988): (0) 3 or more; (1) 1 or 2.
8. Oxalate crystals (including druses) in ovary wall: (0) absent; (1) present.
9. Sclereids in ovary wall: (0) absent; (1) present.
10. Ovule number per carpel: (0) 8 or less; (1) 9 or more.
11. Ovule length at anthesis: (0) 600 μm or less; (1) more than 600 μm.
12. Outer integument, number of cell layers commonly present: (0) 3 or less; (1) more than 3.
13. Inner integument, number of cell layers commonly present: (0) 2; (1) 3.

COMPARATIVE FLORAL ONTOGENY IN WINTERACEAE[1]

Andrew N. Doust[2]

Abstract

The flowers of Winteraceae have often been considered representative of the floral morphology of the earliest angiosperms. The diversity in number and arrangement of the (mostly) free floral organs gives an opportunity to critically examine floral developmental processes in this basal angiosperm family. Comparative ontogeny and morphology of flowers of eight species from six genera of Winteraceae were examined using scanning electron microscopy (SEM) and light microscopy. Results were compared with the floral morphology of *Takhtajania perrieri* (Capuron) Baranova & J-F. Leroy, the recently rediscovered species of Winteraceae from Madagascar. The analysis showed that the basic floral architecture in the family is one of varying numbers of decussate organs followed by whorls with four (or more) organs. Spiral and irregular patterns also occur, with irregularities in floral organ arrangement often the result of initiation of primordia on an asymmetrical floral meristem. The first organs initiated are a pair of sepals that are usually lateral relative to the subtending bract of the flower. Further growth of the calyx is via a ring of meristematic tissue encircling the floral meristem, producing a tubular calyptra bearing the sepals at its apex. The calyptra either encloses the bud until anthesis (*Tasmannia* and *Drimys*) or ruptures while the bud is still young (*Zygogynum*, *Exospermum*, *Bubbia*, *Pseudowintera*, and *Takhtajania*). Differences in the position of the sepals relative to the subtending bract and the position of the petals relative to the sepals create differences in floral architecture between taxa. These differences support *Tasmannia* and *Drimys* being considered separate taxa and *Zygogynum* and *Exospermum* being considered separate from *Bubbia*. The decussate and whorled floral architecture of *Takhtajania perrieri* reflects the basic pattern found throughout the family.

Key words: floral morphology, floral ontogeny, *Takhtajania*, Winteraceae.

Winteraceae have long been considered to be one of the basal angiosperm families and have played an important role in our understanding of the evolutionary history of angiosperms (Bessey, 1915; Bailey & Nast, 1945; Cronquist, 1988). Their importance can be traced to the possession of a suite of morphological character states that have been presumed to be primitive for flowering plants. Foremost among these has been a simple flower construction of many free parts and the occurrence, at least in some taxa, of conduplicate carpels (Bailey & Nast, 1943). Conduplicate carpels reflect the theory that angiosperms evolved carpels by the folding of an ovule-bearing leaf (Bessey, 1915; Bailey & Nast, 1945; Cronquist, 1988). Tubular (ascidiate) carpels can also be found in Winteraceae (Tucker, 1959; Leinfellner, 1965, 1966; Frame-Purguy, 1996).

In recent angiosperm phylogenies Winteraceae are one of the first branching lineages (Qiu et al., 1993; Nandi et al., 1998), and the floral morphology of the family possesses many characteristics that may elucidate the features of floral evolution in basal angiosperms. The recent rediscovery of the only Winteraceae from Madagascar, *Takhtajania perrieri* (Schatz et al., 1998), allows examination of all extant genera in the family.

Other papers in this issue of the *Annals* discuss the phylogenetic placement of *Takhtajania* within Winteraceae and detail its floral and vegetative morphology. Its floral morphology is unusual in the family because of the unique syncarpellate gynoecium (Leroy, 1977), and recent collections have allowed the structure of the gynoecium to be comprehensively examined for the first time (Endress et al., 2000). As a complement to the detailed analysis of *Takhtajania* (other papers, this issue), floral morphology and ontogeny in *Zygogynum bailloni*, *Bubbia howeana*, *Pseudowintera axillaris*, *Pseudowintera colorata*, *Tasmannia lanceolata*, *Tasmannia xerophila*, and *Drimys winteri* are presented. Analysis of the mature floral morphology of other species of *Zygogynum*, *Bubbia*, and *Exospermum* is also presented.

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Comparative analysis of ontogenetic data can help in assessing the possible homologies of mature floral structures. Data from floral ontogeny may also shed light on two persistent systematic questions within the family. One of these is whether the genera *Bubbia, Belliolum, Exospermum,* and *Zygogynum* are best regarded as separate genera or as one large and variable genus. *Bubbia* and *Belliolum* were combined by Burtt (1936). Vink (1985) combined *Bubbia* (including *Belliolum*), *Exospermum,* and *Zygogynum* into an expanded *Zygogynum* s.l., with the argument that the characters that separate the genera (degree of connation of petals and of carpels) were variable and overlapping between them. In this paper data have been recorded under *Bubbia* (including *Belliolum*), *Exospermum,* and *Zygogynum* s. str. so as to highlight possible distinctions between them. The other systematic question is whether *Tasmannia* should be considered a separate genus or a section of *Drimys* (Smith, 1969; Vink, 1970, 1988; Sampson et al., 1988). In this paper data on *Tasmannia* are presented separately from that for *Drimys.*

These results are part of a more comprehensive study of floral development in Winteraceae (Doust, 1997; Doust & Drinnan, 1999; unpublished data). The focus in this paper is on early ontogenetic patterns, especially of sepal and petal initiation.

Materials and Methods

Freshly collected buds and flowers were either dissected immediately or fixed in FAA (formalin, acetic acid, 70% ethanol, 10:5:85 v/v) and stored in 70% ethanol. Preparatory to dissection fixed buds were dehydrated in an ethanol concentration series (70%, 85%, 95%) and stained for greater visibility with 1% acid fuchsin in 95% ethanol. Excess stain was removed from the buds by daily washing in 95% ethanol for three days before dissection. Several changes of 100% ethanol were used to rid the specimens of all water before critical point drying. After drying, specimens were mounted on aluminum stubs and sputter-coated with gold before being imaged in either a Jeol 840 or Phillips XL30 scanning electron microscope. Herbarium specimens of a number of species of *Zygogynum, Exospermum,* and *Bubbia* were examined at MO. The number of buds and flowers dissected and a list of voucher specimens are given in Table 1.

Results

The floral ontogeny and morphology of each species examined are described below. A generalized diagram of the pattern of floral morphology for each species is given in Figure 1.

ZYGOGYNUM BAILLONI

The inflorescence of *Zygogynum bailloni* is one- to three-flowered. Both terminal and lateral flowers have a tubular calycine calyptra, which encircles the young bud but ruptures as the bud expands. The remains of this calyptra are thereafter persistent around the base of the flower. The calyptra bears two sepal tips, which are oriented at right angles to the subtending floral bract in lateral flowers but which are variably oriented in terminal flowers (Fig. 2A). The next organs to be initiated are two opposite decussate sets of petals, the four bases of which are valvate and connate while the tips are free and imbricate The tips of the first initiated pair of these two pairs of petals overlap those of the second. The first pair of petals in lateral flowers is usually oriented parallel (rarely perpendicular) to the orientation of the lateral sepals (Fig. 2A). It was not possible to discern the orientation of the first petal pair relative to the sepals in terminal flowers. The two pairs of decussate petals become connate basally as they grow. They may also be more or less fused post-genitally toward the apices of the petals. An adaxial groove is formed at the boundary between the connate regions of adjacent petals because the adjoining tissue is thinner than the petals themselves. When the petal tips are dissected away from the flower an invagination of the adaxial epidermis into the groove can be seen; epidermal tissue is also clearly evident when the boundary region is observed on the longitudinal edge of an individual petal (Fig. 2B, C). The four outer petals rupture along the boundary grooves when the flower expands at anthesis.

Following the initiation and growth of the outer two pairs of petals, two inner tetramerous whorls of petals are initiated (Fig. 2B). On rare occasions fewer or greater numbers of petals may be found in these whorls. The petals in both inner whorls have narrow bases and grow throughout as completely free structures. The arrangement of the petals is mostly regular, although the sizes of the petal primordia in the inner two whorls vary, apparently according to the space available for them. The second inner whorl of petal primordia is initiated almost at the same height on the meristem as the first inner whorl, and, with continued expansion of the meristem, members of the two whorls rapidly become indistinguishable and the petal bases appear to be in one whorl of eight. However, the middle whorl completely overlaps the inner whorl at its apex.

Table 1. Sample size and voucher list.

Taxon	Sample size (# buds, flowers)	Selected voucher specimens
Zygogynum bailloni Tiegh.	50	NEW CALEDONIA. Rivière Bleue, Dec. 1996, *Doust 927* (MELU), 5 Dec. 1967, *Sampson* (WELTU), 7 Dec. 1967, *Sampson* (WELTU).
Zygogynum bicolor Tiegh.	50	NEW CALEDONIA. Near top of track from Sarraméa to Plateau de Dogny, Dec. 1996, *Doust 926* (MELU); Old lumber road S of road leading from Cascade de Ciu to Koindé and La Foa, 10 Dec. 1993, *McPherson 6121* (MO).
Zygogynum pomiferum Baill.	30	NEW CALEDONIA. Forested slopes of watershed of Rivière des Pirogues, N of Nouméa-Yate road, 15 July 1981, *McPherson 3945* (MO); Thy River valley, ca. 12 air km NE of Nouméa, *McPherson 3082* (MO).
Zygogynum veillardii Baill.	30	NEW CALEDONIA. South of Thio on coastal road, between Nimbo and Camboui Rivers, 26 Apr. 1984, *McPherson 6517* (MO); road N of Canala to Prokoméo and Kouaoua, 20 Apr. 1983, *McPherson 5621* (MO); Prokoméo Region, N of Canala, 28 Dec. 1983, *McPherson 6222* (MO).
Bubbia howeana (F. Muell.) Tiegh.	50	AUSTRALIA. Lord Howe Island: 2 Nov. 1963, *Green* (WELTU), 8 Dec. 1968, *Chinnock* (WELTU).
Bubbia amplexicaulis (Parm.) Dandy	30	NEW CALEDONIA. Mt Panié, ca. 20 air km NW of Hienghéne, 31 Mar. 1981, *McPherson 3695* (MO); Mandjélia, above Pouébo, 30 Jan. 1984, *McPherson 6280* (MO); Pouébo: Mont Mandjélia, 6 Mar. 1979, *MacKee 36654* (MO).
Bubbia comptonii (E. G. Baker) Dandy	30	NEW CALEDONIA. Ponerihouen: Mont Aoupinié, 8 Sep. 1976, *MacKee 31875* (MO); Mt Panié, ca. 20 air km NW of Hienghéne, 20 July 1980, *McPherson 2882* (MO); along old lumber road to top of Mt. Me Ori, above Katrikoin, 6 Sep. 1980, *McPherson 3045* (MO).
Bubbia crassifolia (Baill.) Burtt	30	NEW CALEDONIA. Massif de Boulinda, ca. 8 air km N of Poya, 22 May 1980, *McPherson 2692* (MO); Mont Taom: Crête Est, *MacKee 38150* (MO).
Bubbia pancheri (Baill.) Burtt	30	NEW CALEDONIA. Haute Amoa, 9 Oct. 1971, *MacKee 24400* (MO); along trail from Sarraméa toward Plateau de Dogny, 1 Sep. 1982, *McPherson 4923* (MO); *Duretto 1995* (MEL).
Exospermum stipitatum (Baill.) Tiegh. ex Morot	30	NEW CALEDONIA. Mt Panié, N of Hienghéne, 3 Nov. 1983, *McPherson 5929* (MO); Boguen River Valley, along lumber track above Katrikoin, ca. 20 air km E of Bourail, 21 Nov. 1979, *McPherson 2110* (MO); Tchamba River valley, 1 Nov. 1983, *McPherson 5916* (MO).
Pseudowintera axillaris (J. R. & G. Forst.) Dandy	100	NEW ZEALAND. **North Island**: Kaitoki Waterworks Reserve, Wellington, 3 Nov. 1996, *Doust 891* (MELU); Akatawara Road, Upper Hutt Valley, 4 Nov. 1996, *Doust 892, 894, 895* (MELU); Kakanui Ridge Road, S. Tararuas, 4 Nov. 1996, *Doust 895* (MELU).
Pseudowintera colorata (Raoul) Dandy	100	NEW ZEALAND. **North Island**: Kakanui Ridge Road, S. Tararuas, 4 Nov. 1996, *Doust 896, 897, 899* (MELU); S. Tararuas, 1995, *Sampson* (WELTU).
Tasmannia lanceolata (Poir.) A. C. Smith	200	AUSTRALIA. **Victoria**: Bemm River, East Gippsland, 5 Aug. 1995, *Doust 832* (MELU); Mt. Macedon, 23 Dec. 1996, *Doust 932* (MELU); Mt. Donna Buang, Yarra Ranges National Park, Nov. 1997, *Doust 994–1007* (MELU).
Tasmannia xerophila (P. Parm.) Gray	200	AUSTRALIA. **Victoria**: Mt. Donna Buang, Yarra Ranges National Park, Nov. 1997, *Doust 984–993* (MELU); Lake Mtn., May 1995, *Doust 782* (MELU).
Tasmannia stipitata (Vickery) A. C. Smith	50	AUSTRALIA. **New South Wales**: Point Lookout, 30 Aug. 1981, Sampson & Foreman (NSW); Point Lookout, 15 Sep. 1985, *Sampson & Williams* (NSW).
Tasmannia glaucifolia J. B. Williams	50	AUSTRALIA. **New South Wales**: Dilgry, 28 Nov. 1983, *Williams* (NSW); Moffat Falls, 8 Nov. 1986, *Sampson* (NSW).

Table 1. Continued.

Taxon	Sample size (# buds, flowers)	Selected voucher specimens
Tasmannia insipida R.Br. ex DC.	50	AUSTRALIA. **New South Wales**: Bowens Creek, Blue Mtns., 29 Aug. 1965, *Sampson* (NSW); Doyles River, 1 Sep. 1981, *Sampson & Foreman* (NSW).
Drimys winteri J. R. & G. Forst. var. *chilensis* (DC.) A. Gray	500	AUSTRALIA. **Victoria**: grounds of the University of Melbourne, near Zoology building, Oct. 1998, *Doust 1127–1130* (MELU) (cult.).
Takhtajania perrieri (Capuron) Baranova & J-F. Leroy	20	MADAGASCAR. **Préfecture Antalcha**: Sous-préfecture Andapa, Commune Bealampona, Fokontany Befingotra. Ex village Andranotsarabe, piste vers Ambatomainty suivant une crête, le pic d'Anjanaharibe, *Rakotomalaza 1342* (MO).

Stamen initiation follows directly on from petal initiation. There are usually eight stamens in each whorl, stamens in the first whorl alternating with the whorl of eight petal bases formed from the two inner whorls of petals (Fig. 2D). Succeeding whorls of stamens alternate with each preceding whorl, and there are usually the same number of stamens in each whorl. The last whorl of organs initiated contains both stamens and carpels, and the larger size of the carpels often leads to a reduction in the total number of organs in this whorl (Fig. 2D). The carpels are connate but there are no connections between the stigmatic crests and thus no common pollen-transmitting tract (Igersheim & Endress, 1997).

BUBBIA HOWEANA

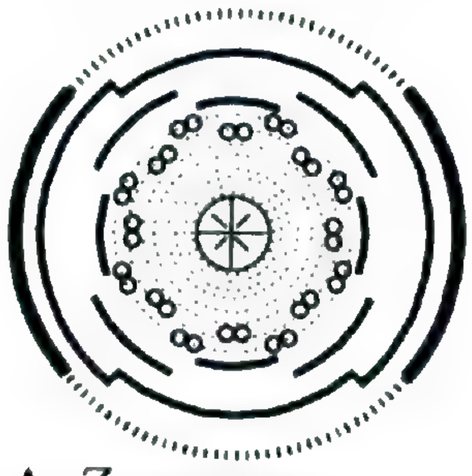
Each flower of *Bubbia howeana* has a calycine calyptra that protects the bud early in development but which ruptures as the bud expands. The remains of the calyptra persist as remnants of tissue around the base of the flower. The calyptra initiates as two laterally placed free sepals but attains most of its growth by the action of an annular meristem underneath the sepal tips. Two outer decussate pairs of petals are initiated next, with the first of these two pairs of petals oriented perpendicularly to the sepal tips. The two outer decussate pairs of petals in the flowers of *B. howeana* are free throughout their growth, and they tightly wrap around the bud and protect it as it develops (Fig. 2E). An inner whorl of petals is next initiated with four or rarely five petals, followed by a second whorl of petals and/or stamens (this whorl is shown as the outermost whorl of stamens in Fig. 1B). Three or four alternating whorls of four or five stamens are then initiated, followed by three to six free carpels arranged either symmetrically or more irregularly.

PERIANTH PATTERN IN *ZYGOGYNUM* AND *BUBBIA*

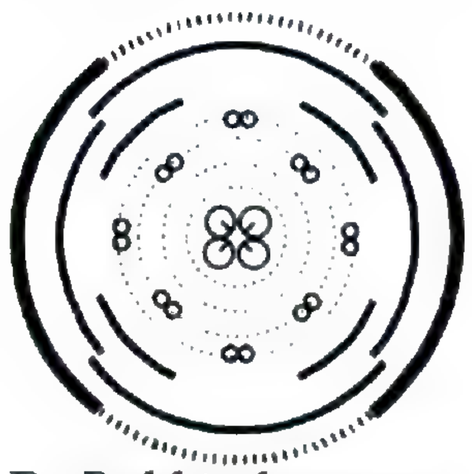
There is a difference in orientation of sepals and petals in *Zygogynum bailloni* and *Bubbia howeana*. In *Zygogynum bailloni* the lateral sepals are followed by a pair of lateral petals (Figs. 1A, 2A), whereas in *Bubbia howeana* the lateral sepals are followed by a pair of medial petals (Figs. 1B, 2E). These contrasting patterns of perianth morphology were examined in herbarium specimens of a number of species of *Zygogynum*, *Bubbia*, and the related genus *Exospermum* (Vink, 1985), and the results are given in Table 2. There is a clear distinction in perianth pattern between *Bubbia* on the one hand and *Zygogynum* and *Exospermum* on the other, with lateral flowers of all species of *Bubbia* examined having lateral sepals and medial first petal pair and lateral flowers of all species of *Zygogynum* and *Exospermum* examined having lateral sepals and lateral first petal pair. The two groups of species examined also differ in petal connation, with species of *Bubbia* having free outer petals and species of *Zygogynum* and *Exospermum* (except *Z. pomiferum* and some specimens of *E. stipitatum*) having connate outer petals.

PSEUDOWINTERA AXILLARIS AND *P. COLORATA*

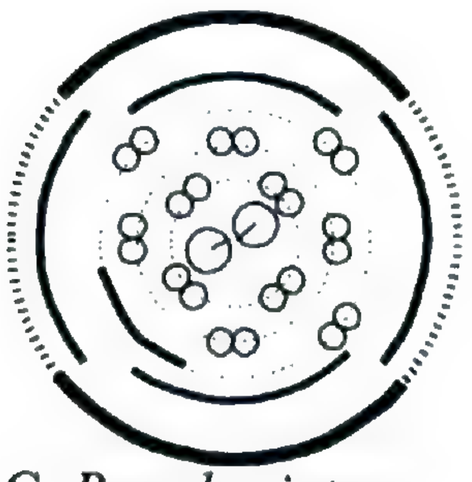
No material of the earliest developmental stages for either species of *Pseudowintera* was examined. However, at a somewhat later stage the buds are enclosed in a calycine calyptra that has two lateral sepal tips. F. B. Sampson (pers. comm.) and Vink (1970) reported that three sepal tips may be present. The calyptra encloses the bud during early development but ceases growth while the bud is still small. After the calyptra is ruptured by the expansion of the developing bud two or three lobes persist around the base of the flower. The pattern of initiation of the petals is somewhat variable, and



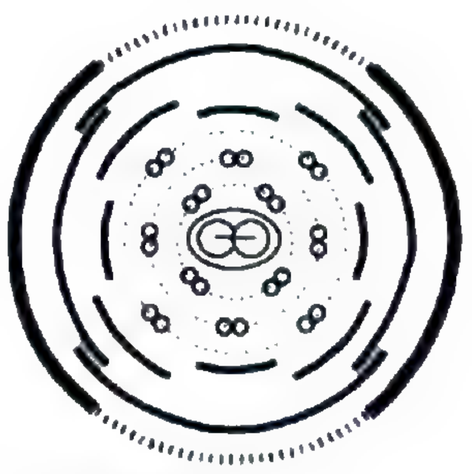
A. *Zygogynum bailloni*



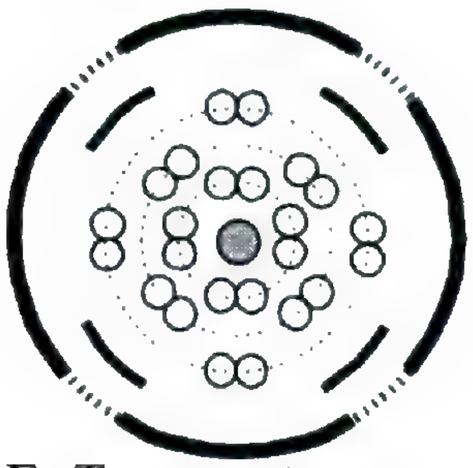
B. *Bubbia howeana*



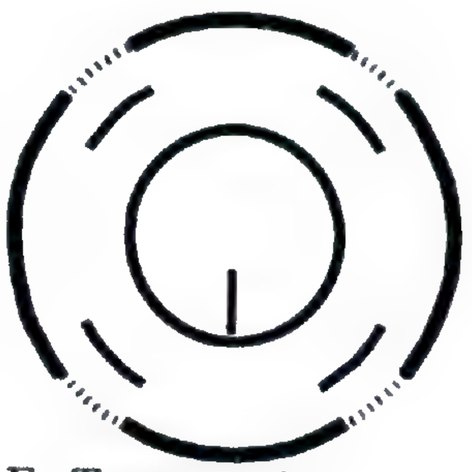
C. *Pseudowintera colorata*



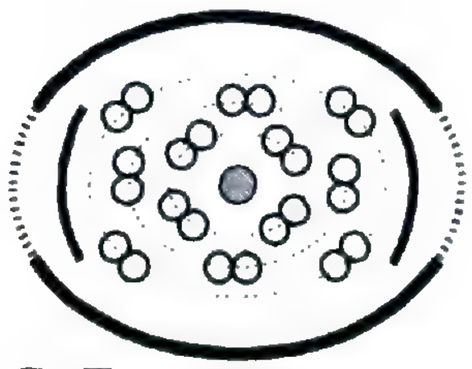
D. *Takhtajania perrieri*



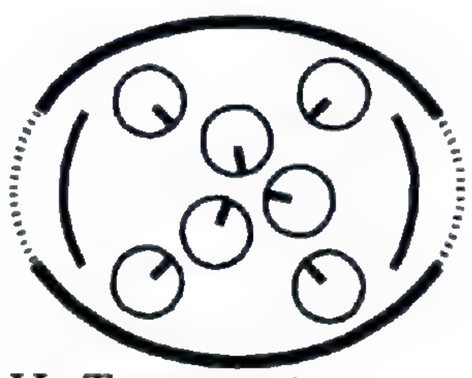
E. *Tasmannia lanceolata* (male)



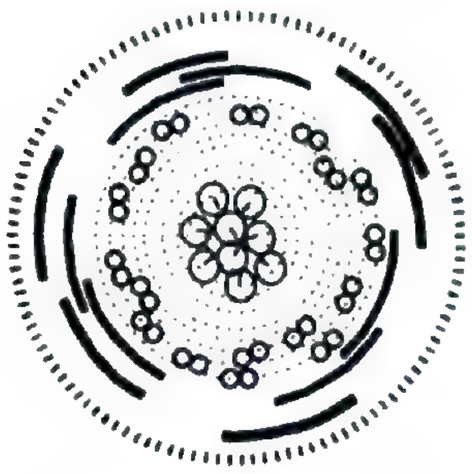
F. *Tasmannia lanceolata* (female)



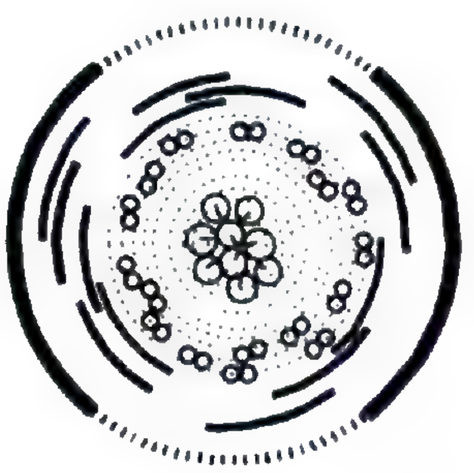
G. *Tasmannia xerophila* (male)



H. *Tasmannia xerophila* (female)



I. *Drimys winteri* (terminal)



J. *Drimys winteri* (lateral)

Key:

differences in size of the petals may indicate that they are initiated sequentially (Fig. 2F). However, the petals are often in two decussate pairs with further petals in a whorl inside these first four petals. Other patterns, such as spirals and whorls of three, are also occasionally seen.

TASMANNIA LANCEOLATA AND *T. XEROPHILA*

The plants of *Tasmannia* are dioecious, but the pattern of sepal and petal initiation is the same in both male and female flowers (although the number of organs can differ). In *Tasmannia lanceolata* two sepals are initiated at the lateral poles of the meristem, followed by the initiation of two more sepals situated medially (adaxially and abaxially) (Fig. 3A, B). The abaxial sepal is at first only a line of tissue, but a flap develops from this region in later developmental stages. In *Tasmannia xerophila* and all other species of *Tasmannia* examined (Table 1) only medial sepals are initiated, the adaxial sepal initiating before the abaxial sepal (Fig. 3D, E). The continued growth of the calyx in both species occurs via a ring of meristematic tissue that encircles the floral meristem, so that the calycine calyptra grows as a cylinder, bearing the sepal tips at its apex. The calyptra encloses the bud until anthesis, when it ruptures and abscises as the flower expands.

In all species of *Tasmannia* petals are initiated soon after the sepals and alternate with them. In *Tasmannia lanceolata* four petals alternate with the four sepals (Fig. 3B), while in other species of *Tasmannia* two lateral petals initiate perpendicular to the two medially placed sepals (Fig. 3E). In all species further petals may be initiated alternating with the previous whorls. (Note that in *Drimys piperita* [*Tasmannia*] s.l. [as defined by Vink, 1970] staminate and pistillate flowers of the same entity [species] may have different numbers of petals [P. F. Stevens, pers. comm.]). In staminate flowers in *Tasmannia lanceolata* and *T. xerophila* stamen initiation follows directly on from the initiation of the petals (Fig. 3C). The arrangement of the stamens may be variable, as the shape of the meristem is often asymmetric. A single sterile carpel is the last organ to be initiated. In pistillate flowers of *T. lanceolata* a single terminal carpel is initiated after the initiation of the petals, while in other species a number of lateral carpels are usually initiated (*T. insipida* only initiates one or occasionally two carpels). The position of the first four of these lateral carpels alternates with the positions of the medial sepals and lateral petals (Fig. 3F). When more than two petals are initiated these alternate with the "whorl" of the medial sepals and lateral petals in the same positions as illustrated for the first four carpels (Fig. 1H).

DRIMYS WINTERI

The inflorescences of *Drimys winteri* are composed of a number of racemose uniflorescences with a terminal and up to eight lateral flowers. In terminal flowers the calycine calyptra initiates as a ring of tissue around the approximately circular floral meristem (Fig. 4A), whereas in lateral flowers two sepal tips are initiated laterally with respect to the subtending floral bract (Fig. 4B). These sepal tips are subsequently borne aloft by the growth of a calycine ring of tissue around the floral meristem, so that the growth of the calyptra is similar in terminal and lateral flowers. The calyptra eventually encloses the developing flower, and at anthesis the calyptra splits into two or three segments and the petals unfold. The reflexed calyptra segments eventually wither and abscise.

In both terminal and lateral flowers there is a considerable delay between initiation of the calycine calyptra and the corolla; petal primordia appear only when the calyptra has almost enclosed the floral meristem. At this stage the floral meristem is large and domed, and petals initiate low down on the flank of the dome (Fig. 4C). The initiation of stamens follows the petals without delay. A number of tiers of stamens are initiated on the almost vertical flanks of the floral meristem. The initiation of carpels follows the stamens without delay and, as the growth of the floral meristem ceases, the carpels eventually develop all over the flattened apex of the dome (Fig. 4D). The arrangement of the pet-

←

Figure 1. Generalized floral diagrams for each of the taxa examined. These diagrams give only the main outline of morphological patterns, as much variation can exist in the pattern of floral initiation for any species. In all diagrams the axis is toward the top of the diagram and the subtending floral bract is toward the bottom. Organs positioned laterally are toward the sides of the diagrams, and organs positioned medially are at the top and bottom of the diagrams. Many variations on these patterns can be found, especially in *Drimys*, *Pseudowintera*, and in the numbers of stamens and carpels in all taxa. Where there are numerous whorls of stamens only the first two or three whorls have been shown and the others have been indicated by broken circles. Drawings are not drawn to scale relative to each other.

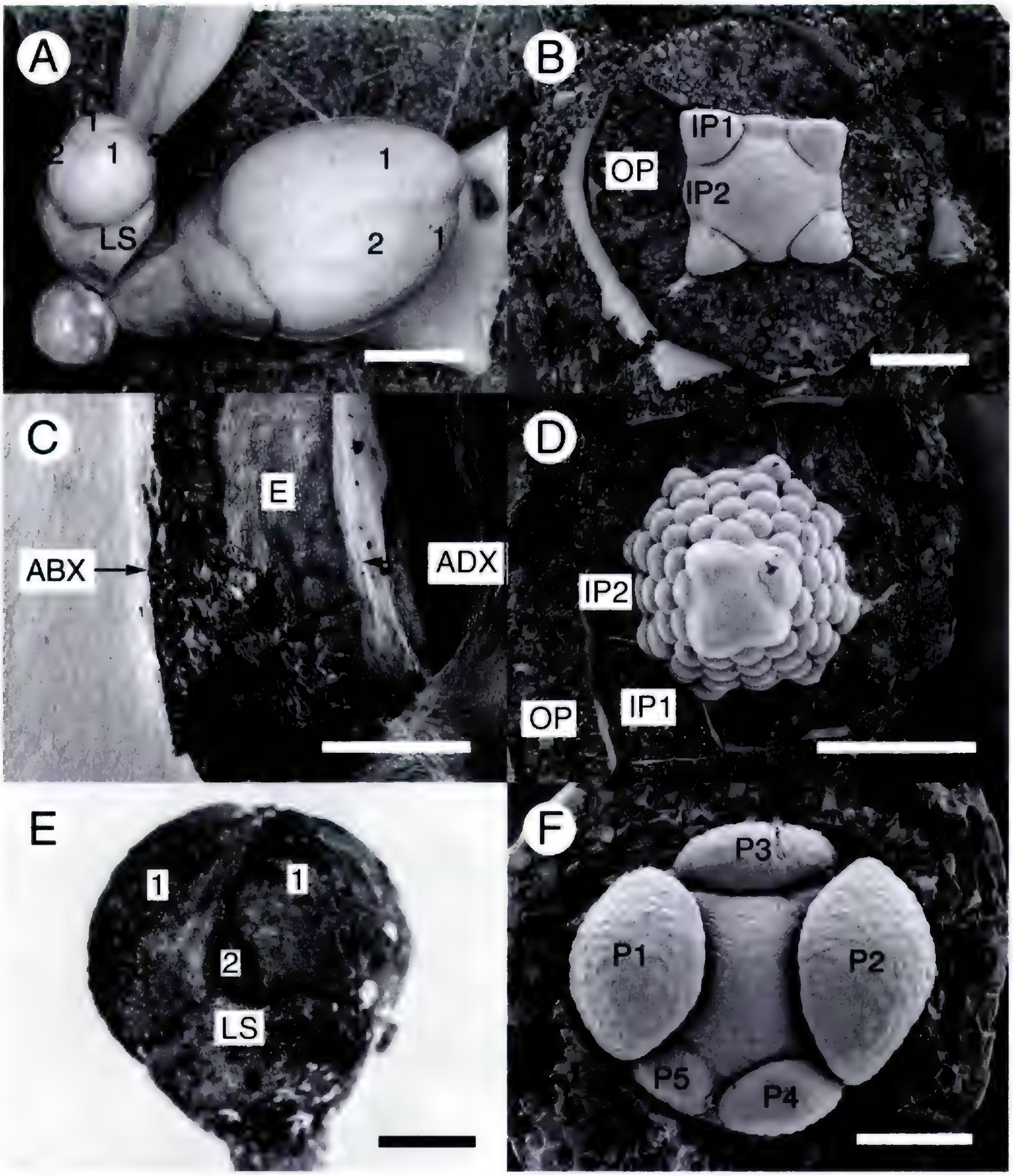


Figure 2. —A. *Zygogynum bailloni*: a terminal and lateral bud, with the lateral bud (left) showing the lateral position of both the sepal tip (LS) and the first pair of outer petals (1). The second pair of outer petals, perpendicular in position to the first pair, is also visible (2). In the terminal bud the position of the first and second pair of outer petals relative to the sepal tips is not clear. Scale = 5 mm. —B. *Z. bailloni*: a young bud with calyptra and outer four petals removed. The invagination of the adaxial epidermis between the scars of each of the outer four petals (OP) can easily be seen. The two alternating whorls of inner petals (IP1, IP2) have just been initiated. Scale = 200 μm. —C. *Z. bailloni*: a longitudinal view of the basal region of the edge of an outer petal showing the smooth covering of epidermis (E) on the adaxial side (ADX) of the petal and the broken cells of the connate portion of the petal toward the abaxial side (ABX). Scale = 500 μm. —D. *Z. bailloni*: late bud stage with calyptra and petals removed, showing regular whorls of stamens and the somewhat irregular arrangement of the carpels as they begin to enlarge (outer petal scars = OP, scars of first whorl of inner petals = IP1, scars of second whorl of inner petals = IP2). Scale = 500 μm. —E. *Bubbia howeana*: side view of a bud showing the lateral sepal tip (LS) and the first pair of outer petals (1) oriented perpendicular to the sepal tips. A small section of one of the second pair of outer petals (2) is also visible. Scale = 1.5 mm. —F. *Pseudowintera colorata*: a young bud with the calycine calyptra removed showing the arrangement of the five petals. Petals 1 and 2 (P1, P2) are opposite each other and petal 3 (P3) is alternate with these two. Petal 4 (P4) is almost opposite petal 3 but petal 5 (P5) appears to be either transitional to a spiral pattern or in the first position of the next whorl. Scale = 100 μm.

Table 2. Perianth pattern and petal connation in some species of *Zygogynum*, *Exospermum*, and *Bubbia*. These were observed from the fresh and herbarium specimens detailed in Table 1. Positions noted for the sepals and first pair of petals are relative to the subtending floral bract.

Taxon	Position of sepals	Position of first pair of petals	Petal connation
Zygogynum bailloni	lateral	lateral	connate
Zygogynum bicolor	lateral	lateral	connate
Zygogynum veillardii	lateral	lateral	connate
Zygogynum pomiferum	lateral	lateral	(mostly) free
Exospermum stipitatum	lateral	lateral	(mostly) connate
Bubbia howeana	lateral	medial	free
Bubbia amplexicaulis	lateral	medial	free
Bubbia comptonii	lateral	medial	free
Bubbia crassifolia	lateral	medial (rarely lateral)	free
Bubbia pancheri	lateral	medial	free

als, stamens, and carpels may either be in more or less regular whorls or spirals.

TAKHTAJANIA PERRIERI

The flower of *Takhtajania perrieri* has a two-lobed calycine calyptra with the lobes oriented laterally with respect to the subtending floral bract. Although no young buds at early stages were available it is likely that the calycine calyptra initially encloses the bud before rupturing as the bud increases in size. Fragments of the calycine calyptra are persistent around the floral base in flowering and fruiting stages. The initiation of this calyptra is followed by initiation of the outer petals in two decussate pairs. After the initiation of the outer two pairs of petals an inner whorl of four petals is initiated, the positions of the inner petals alternating with the positions of the four outer petals. This is followed by the initiation of a further tetramerous whorl of petals. The outer petal pairs are imbricate and fused to each other along their lateral overlapping edges (as shown by Endress et al., 2000), and the basal adaxial face of the outer petals is partially fused to the abaxial faces of the petals in the first inner whorl of petals (Fig. 4E, F). Occasionally more than four petals may occur in each of the inner two whorls of petals; sometimes this is because the fifth petal is in the position of one of the stamens of the first androecial whorl. Usually two complete whorls of four or five stamens are initiated, as well as one or two stamens of a third whorl before initiation of the gynoecium. The syncarpous gynoecium is terminal, and its morphology is fully described by Endress et al. (2000). The overall structure of the flower comprises three sets of opposite decussate whorls followed by alternating tetramerous or pentamerous whorls, and ending in the formation of the terminal gynoecium.

Discussion

SEPAL INITIATION

In all species of Winteraceae the calyx forms a tubular calyptra, which completely encloses the bud for at least a short time during development. The presence of the calyptra appears to be a synapomorphy for Winteraceae, as neither of the likely outgroups of Canellaceae or Degeneriaceae (Suh et al., 1993; Nandi et al., 1998; Karol et al., 2000; Endress et al., 2000) possess such a tubular structure enclosing the bud.

In *Drimys winteri*, *Tasmannia lanceolata*, and *T. xerophila* the calyptra grows with and encloses the bud until anthesis, abcising as the flower opens. However, in *Zygogynum bailloni*, *Bubbia howeana*, *Pseudowintera axillaris*, and *P. colorata* the calyptra encloses the bud only during early development and is ruptured as the bud enlarges. The remnants of the ruptured calyptra persist around the base of the flower and can still be seen in flowering and fruiting stages. Early stages of the flowers of *Takhtajania perrieri* were not available for study, but the mature flower shows remnants of the calyptra around the base of the flower similar to those found in *Zygogynum*, *Bubbia*, and *Pseudowintera*. This implies that in *T. perrieri* the calyptra encloses the bud only in early development. Other species of *Zygogynum*, *Exospermum*, *Bubbia*, and *Pseudowintera* also have a calyptra that encloses the bud only during early development (Sampson, 1963; Vink, 1970, 1977, 1983, 1988).

The first stage in the development of the calyptra in nearly all flowers in the family is the initiation of two or more sepals. The exception is in the terminal flower of *Drimys winteri* (and possibly other species of *Drimys* as well), where the calyx initiates as an annular primordium surrounding

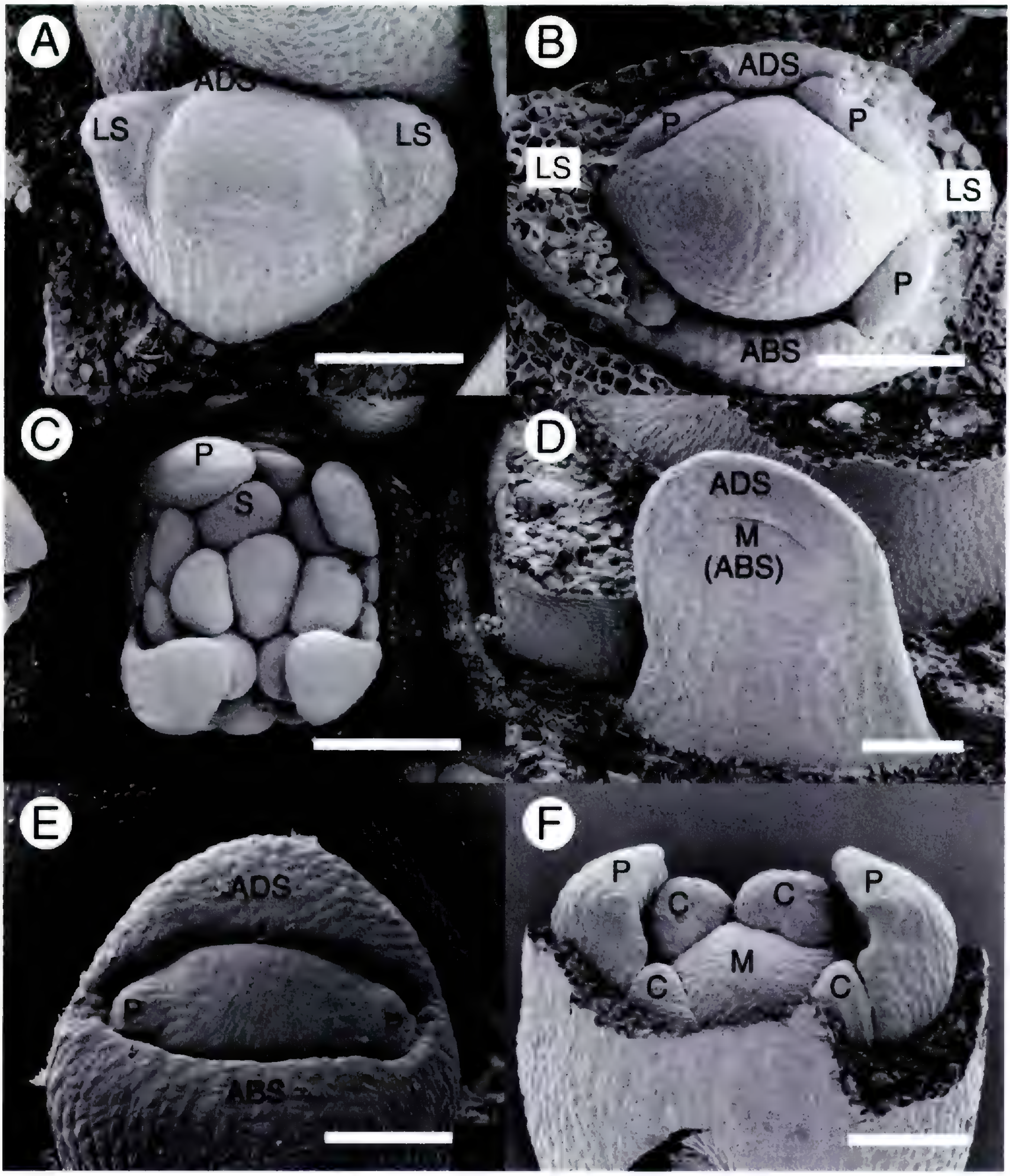


Figure 3. —A. *Tasmannia lanceolata*: two lateral sepals (LS) have been initiated, and the adaxial sepal (ADS) can just be seen. The abaxial sepal at this stage is typically merely a line of tissue. Scale = 100 μm. —B. *T. lanceolata*: petals (P) are being initiated in positions alternate with the lateral (LS) and medial (adaxial = ADS, abaxial = ABS) pairs of sepals. Scale = 100 μm. —C. *T. lanceolata*: semi-mature bud with calyptra removed showing the initiation of four petals (P) and a number of whorls of stamens (S). A sterile carpel will be the last organ to be initiated. Scale = 200 μm. —D. *Tasmannia xerophila*: early sepal initiation with only the adaxial sepal (ADS) and the meristem (M) visible. The position where the abaxial sepal will initiate is marked in parentheses as (ABS). Scale = 100 μm. —E. *T. xerophila*: both adaxial (ADS) and abaxial (ABS) sepals have been initiated and two petals (P) have now been initiated in lateral positions, alternate with the sepals. Scale = 100 μm. —F. *T. xerophila*: a young bud with the sepals removed, showing four carpels (C) that have been initiated in positions alternate with the medial sepals and lateral petals (P). Note the tilted meristem (M). Scale = 100 μm.

the floral meristem. In the lateral flowers of *D. winteri* two sepal tips lateral to the subtending floral bract are commonly initiated before the bulk of the growth of the calyptra takes place via an annular meristem. The initiation of sepals lateral to the subtending floral bract is also seen in lateral flowers of *Zygogynum*, *Exospermum*, *Bubbia*, and *Pseudowintera*. A calyptra also forms in terminal

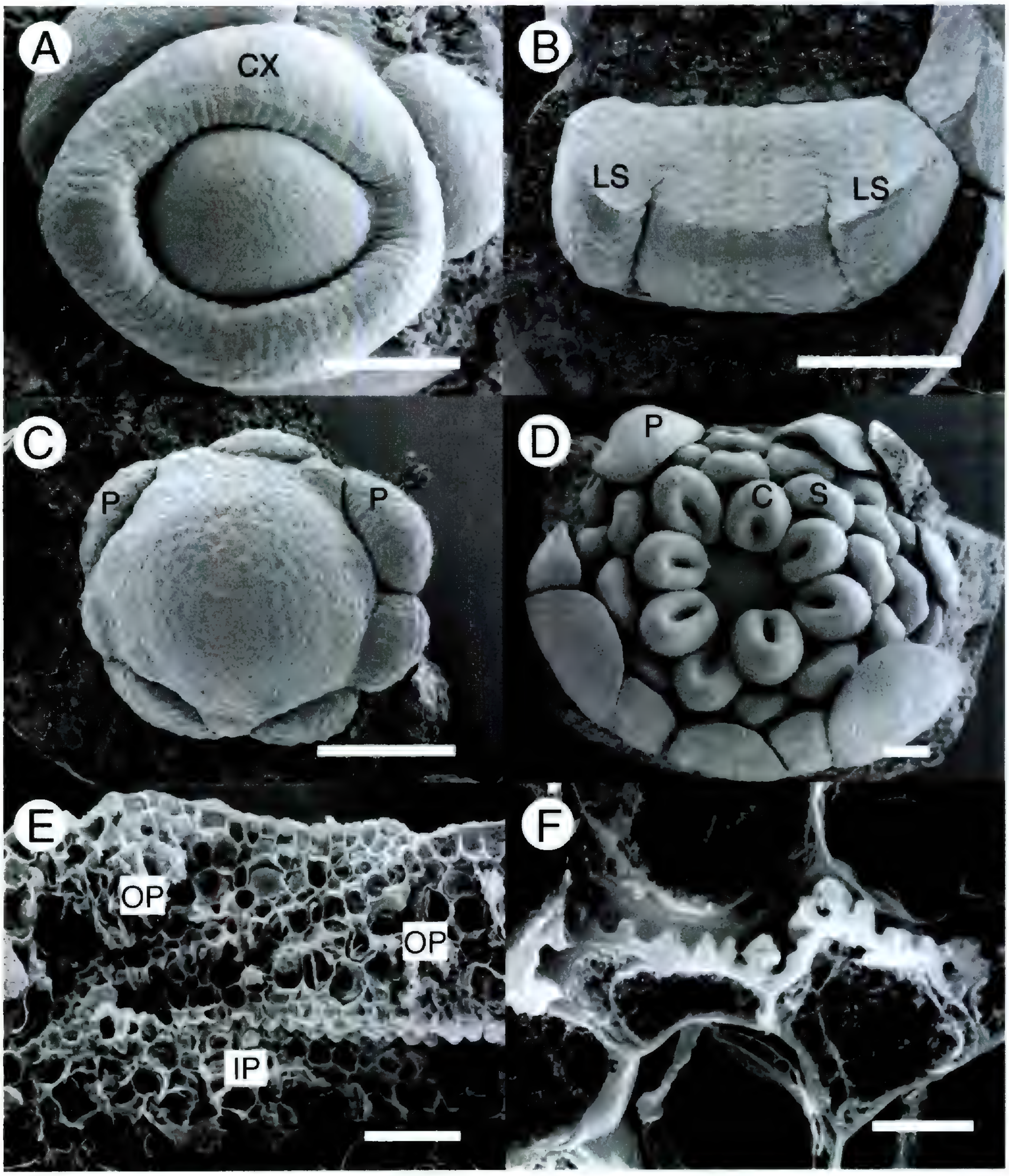


Figure 4. —A. *Drimys winteri*: a terminal flower bud at the stage of early calyx initiation showing the annular calyx primordium (CX). Scale = 100 μm. —B. *D. winteri*: a lateral flower bud showing the two lateral sepals (LS). Scale = 100 μm. —C. *D. winteri*: a lateral bud with the calyx removed showing the initiation of petal primordia (P), with the primordia being in no particular relationship to the arrangement of the sepals. Scale = 100 μm. —D. *D. winteri*: a late stage bud with the calyptra removed, showing the initiation of stamens and carpels. Petals (P), stamens (S), and carpels (C) in this flower are arranged in a spiral pattern. Scale = 100 μm. —E. *Takhtajania perrieri*: a transverse section of the petal region showing fusion of two outer petals (OP) and an inner petal (IP). Scale = 50 μm. —F. *T. perrieri*: a close-up of the region of fusion of the petals (from a different specimen from Fig. 4E) showing interdigitation of the cuticles. Scale = 5 μm.

flowers in these genera, but here the orientation of the sepals is unclear. In *Tasmannia lanceolata* lateral sepals are initiated as well as a further pair of medial sepals. In *T. xerophila* only two medial sepals are initiated. Inspection of semi-mature buds and flowers of *Takhtajania perrieri* indicates that two lateral sepals are initiated. Thus the pattern of initiation of the sepals has three states; two lateral sepals, two medial sepals, or two lateral sepals followed by two medial sepals. By itself,

the pattern of initiation allies *Drimys* with *Takhtajania*, *Zygogynum*, *Bubbia*, and *Pseudowintera*, rather than with *Tasmannia*. The pattern shown by *Tasmannia lanceolata* (two lateral sepals and two medial sepals) is intermediate between the flowers of other *Tasmannia* species and those of the rest of the genera in the family.

The pattern of initiation of the sepals in *Tasmannia* and *Drimys* may help decide whether these two taxa should be considered as one genus or two (Smith, 1943a, b; Vink, 1988; Sampson et al., 1988). Smith (1969) segregated *Tasmannia* as a separate genus from *Drimys* because of differences in chromosome number (*Tasmannia*, $n = 13$, *Drimys*, $n = 43$; Ehrendorfer et al., 1968), and sexuality of the flowers (*Drimys* is bisexual, *Tasmannia* is unisexual and dioecious). The two genera also differ markedly in flavonoid composition (Williams & Harvey, 1982). However, Vink (1970, 1988, 1993) disagreed with the segregation of *Tasmannia*, and recombined it with *Drimys*, citing the calyptra that protects the bud until anthesis, the monopodial construction of both *Tasmannia* and *Drimys*, and the observation that sporadic plants of *Tasmannia* have bisexual flowers. In the molecular phylogenies presented by Suh et al. (1993) and Karol et al. (2000) *Tasmannia* and *Drimys* do not appear as sister taxa, but as sequentially branching lineages on the tree. The differences in position of sepal initiation between the two genera supports the branching pattern of the molecular phylogenies, and is consistent with *Tasmannia* being a separate genus from *Drimys*. This implies that the possession of a calyptra that encloses the bud until anthesis may have evolved independently in these two genera.

FLORAL ORGAN ARRANGEMENT

Flowers in Winteraceae generally have organs arranged in pairs and whorls. In most cases the pattern in the flowers is determined by the position of the sepals, and, in general, succeeding pairs or whorls of organs alternate with the previous pair or whorl. This is the case in *Bubbia* and *Takhtajania*, where a lateral pair of sepals is followed by medial and lateral pairs of petals (Table 2; Figs. 1B, D, 2E). A number of whorls of petals, stamens, and carpels are then initiated. A similar situation occurs in *Zygogynum* and *Exospermum*, except that the sepals and the first pair of outer petals are parallel with each other (they are both lateral with respect to the subtending floral bract) (Table 2; Figs. 1A, 2A). In the large flowers of *Z. bailloni* the two innermost tetramerous whorls of petals initiate almost simultaneously and act as one pseudo-whorl of eight petals (Fig. 2B). Succeeding whorls of eight stamens each alternate with the pseudo-whorl of eight petals (Figs. 1A, 2D). Irregularities in floral organ arrangement are often seen in the larger flowers of species of *Zygogynum* and *Exospermum* (pers. obs.; Endress, 1986). These have previously been explained as the result of the combination of asymmetry of the floral apex and the initiation of small primordia relative to the size of the floral apex (Endress, 1986). Another cause of irregular arrangements is that the changeover from petal to stamen and from stamen to carpel production can occur within one whorl. The different sizes of the primordia in a whorl can then perturb the pattern of primordial position.

The difference in sepal and petal initiation in *Zygogynum* and *Exospermum* (lateral sepals, lateral first pair of petals) versus that in *Bubbia* (lateral sepals, medial first pair of petals) was also noted by Vink (1977). This difference does not support the decision by Vink (1985) to combine *Zygogynum* s. str., *Exospermum*, and *Bubbia* into an expanded *Zygogynum* s.l. Vink made his decision after observing that the main characters used to separate the genera (degree of connation of the petals and carpels) were variable within each genus and transitional between genera (Vink, 1985). Such variability was observed in the present study for species of *Zygogynum* (Table 2) and *Exospermum*. However, the position of the sepals and first pair of petals indicates that the three genera fall into two groups; (*Zygogynum* + *Exospermum*) and (*Bubbia*). This suggests that it might be advantageous to use floral ontogeny to reexamine the homology of petal and carpel connation in the different genera.

A basic pattern of sets of decussate and whorled organs is also found in flowers of *Tasmannia*. There are two distinct variations in this basic pattern of floral organ arrangement within flowers of *Tasmannia*. In *Tasmannia lanceolata* a lateral pair of sepals is initiated followed by a medial pair. A tetramerous whorl of petal primordia is then initiated, with the positions of the four petals alternating with those of the four sepals. Further petals, stamens (male flowers), or a carpel (female flowers) are then initiated. In *Tasmannia xerophila* two sepal primordia are initiated medially, followed by two petal primordia initiated laterally. A whorl of four organs (which may be petals, stamens [male flowers], or carpels [female flowers]) are then initiated alternating with the "whorl" comprised of the pair of sepals and the first pair of petals. More organs can also be initiated. The similarity in floral organ arrangement between the flowers of these two species of *Tasmannia* is that in both, two sets of decussate

organs are followed by a tetramerous whorl of organs. The differences are in the position of the first pair of organs (lateral sepals in *T. lanceolata* vs. medial sepals in *T. xerophila*) and in the identity of the second pair of organs (medial sepals in *T. lanceolata* vs. lateral petals in *T. xerophila*). All other species of *Tasmannia* examined conform to the pattern shown by *T. xerophila*.

The initiation of the perianth in *Pseudowintera* is consistent with the general pattern described above. There is usually at least one pair of decussate organs, the laterally positioned sepals, but there may also be one or two decussate pairs of petals alternate with the sepal tips. Vink (1970) and F. B. Sampson (pers. comm.) reported that three sepal tips may also occur, along with trimerous whorls of petals and stamens, as illustrated by Vink (1970, fig. 3e). A transition to spiral arrangements of the stamens and carpels starting with the innermost petals also has been observed.

The floral arrangement in *Drimys winteri* is unlike that in the other genera because there appears to be no relationship between position of the lateral sepals or calyx and the succeeding petals. The lack of relationship in lateral flowers may be because the delay between initiation of the two organ types may deprive the petals of positional information from the sepals. There is, of course, no positional information given by the calyx in terminal flowers, as the calyx is initiated as an annular primordium encircling the floral meristem. There is also a variety of whorled and spiral floral organ arrangements in *D. winteri*, implying that petal, stamen, and carpel arrangement is not constrained by sepal position. Irregularities in the position of the primordia have been correlated with asymmetry of the floral meristem (unpublished results; Erbar & Leins, 1983).

AFFINITIES OF *TAKHTAJANIA*

Takhtajania perrieri was originally described as a species of *Bubbia* by Capuron (1963), and its floral architecture is similar to that of *Bubbia howeana*. Both species share a decussate pattern of the sepals and first two pairs of petals, followed by a whorl or whorls of petals and several whorls of stamens in fours or fives. However, the basic pattern of decussate pairs of organs followed by whorls of organs has been shown to be common throughout the family, with exceptions being found only in the relationship between the sepals and the first pair of petals in *Zygogynum* (parallel to each other), the terminal flowers of *Drimys winteri* (no decussate organs), the lateral flowers of *D. winteri* (opposite sepals followed by various arrangements of petals, stamens, and carpels), and some flowers of *Pseudowintera* spp. (opposite petals alternating with the sepals but further petals either in whorls or spirals). A basic pattern in the family of four petals alternating with the sepal tips was also postulated by Vink (1988). Thus the similar patterns of floral organ arrangement in *Takhtajania perrieri* and *Bubbia howeana* show the general relationship in the family rather than being evidence of a special shared relationship.

Both *Zygogynum bailloni* and *Takhtajania perrieri* have fused outer petals. Such fusion does not generally occur in the corolla of the flowers of the other genera in the family and may provide evidence of a shared relationship. The method of fusion of outer petals in *Z. bailloni* is both through connation in the basal regions as well as variable amounts of post-genital fusion in the otherwise free apical regions. In the species of *Zygogynum* that display basally connate petals the mode of connation may be the result of coalescence of the margins of the petal primordia as they grow, whereupon the epidermis in the region of connation disappears. However, in *T. perrieri* the petals cohere only through post-genital fusion of the cuticles, the fusion occurring by the interdigitation of the cuticles in the overlapping petal areas (as documented by Endress et al., 2000). Interdigitation of the cuticles was also observed to occur between the bases of the outer petals and the basal portions of the next inner whorl of petals. Post-genital fusion as it occurs in *T. perrieri* may be likened to a cohesion of the petals rather than a true connation as found in the basal portion of the outer petals of *Zygogynum*. This makes it possible that the morphological similarity between the fused outer petals of the two species is not homologous.

CONCLUSIONS

The study of the ontogeny of the flowers of Winteraceae reveals both unifying patterns and unexpected complexity. The underlying pattern of floral organ arrangement is one of sets of decussate and whorled organs, occasionally turning to spirals in *Drimys* and *Pseudowintera*. Differences within the basic pattern include the number and position of sepals and the position of the petals relative to the sepals. In all species irregularities in floral apex symmetry and in the size of organs in a whorl may perturb the regular whorled or spiral pattern, leading to flowers with more irregular floral organ arrangements. There is a clear morphological division in the family between those taxa that retain the

calycine calyptra until anthesis (*Tasmannia* and *Drimys*) vs. those where the calyptra is ruptured early in development (*Bubbia*, *Zygogynum*, *Exospermum*, *Pseudowintera*, and *Takhtajania*). However, differences in the position and number of sepals between *Tasmannia lanceolata*, *T. xerophila*, and *Drimys winteri* suggest that the two genera should not be combined into one. Differences in the position of the first pair of petals relative to the sepals suggest that *Zygogynum* and *Exospermum* are distinct from *Bubbia*. The decussate and whorled arrangement of the floral organs in *Takhtajania perrieri* is closest to that of species of *Bubbia*, but this pattern is common throughout the family and does not serve as evidence of a special relationship between the two taxa. The manner of fusion of the outer petals in *T. perrieri* is strictly post-genital and by interdigitation of the cuticles, whereas that in *Z. bailloni* is primarily by connation of the basal region of the petals. These differences do not support a close relationship between *Takhtajania* and *Zygogynum*. These observations are congruent with the molecular phylogeny of Karol et al. (2000), where *Takhtajania* is basal, and its unique syncarpellate gynoecium autapomorphic.

The ontogenetic data presented in this paper are a step toward understanding the evolution of floral form in Winteraceae. On the basis of robust interpretations of phylogenetic relationships within the family (other papers, this issue), an understanding of the evolution of diverse and variable floral forms within such a well-defined monophyletic group will also advance our understanding of the evolution of the flower in basal angiosperms.

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THE POLLEN OF *TAKHTAJANIA PERRIERI* (WINTERACEAE)[1]

F. B. Sampson[2]

Abstract

Pollen morphology and ultrastructure of *Takhtajania perrieri* (Capuron) Baranova & J.-F. Leroy, the Malagasy member of the Winteraceae, were studied and compared with pollen of other members of this primitive angiosperm family. Pollen is in permanent tetrahedral tetrads, which are the largest in the family. As in *Drimys* and *Pseudowintera*, the apertures are expanded into protuberant papillae, suggesting premature commencement of growth of the pollen tubes. Apertures are circular or slightly oval, and it is concluded that previous reports of colpate and trichotomocolpate (trichotomosulcate) apertures, based on the study of dried material from the type specimen, are incorrect. The exine is reticulate and tectate-columellate and most columellae have characteristic expanded bases where they join the foot layer. Beneath the foot layer is an endexine and an intine, which is two-layered in apertural areas, as in other Winteraceae. As in some other genera, the aperture is surrounded by an annulus, with an underlying thickened endexine consisting of tangentially aligned lamellae. The septal exine is highly reduced, and small gaps in the ectexine are plugged with endexine, suggesting that pollen mitosis is asynchronous within a tetrad, as in *Pseudowintera* and *Drimys*, but in contrast to *Belliolum*, *Bubbia*, *Exospermum*, *Tasmannia*, and *Zygogynum*, in which these gaps remain or are blocked by the deposition of intine after pollen mitosis. On the basis of its pollen morphology, *Takhtajania* seems most closely related to *Drimys* and *Pseudowintera*.

Key words: *Bubbia perrieri*, flora of Madagascar, Magnoliidae, pollen morphology, *Takhtajania*, tetrad pollen, Winteraceae.

The rediscovery of *Takhtajania perrieri* (Schatz et al., 1998) has provided the opportunity to study liquid-preserved polliniferous material for the first time. Although there have been three quite detailed studies of the pollen of this plant (Straka, 1963; Lobreau-Callen, 1977; Praglowski, 1979), they were based on dried material from the type specimen, collected nearly 90 years ago. These three authors disagreed on the interpretation of some aspects of the pollen of *Takhtajania*.

Takhtajania pollen is in permanent tetrahedral tetrads, a feature that occurs in all other members of the Winteraceae, with the exception of four species of *Zygogynum*, which have monads (Sampson, 1974; Praglowski, 1979; Vink, 1993). Monad pollen in these *Zygogynum* species seems to have evolved from the tetrad condition (Sampson, 1981; Doyle et al., 1990a, b). Previous studies on the pollen of *Takhtajania* indicated that its pollen tetrads are larger than those of other Winteraceae. It has been claimed, too, that a few apertures of *Takhtajania* are of the trichotomosulcate type, with a three-slit sulcoid aperture, in contrast to the invariably round or oval apertures of other members of the family.

The present study was undertaken, therefore, to provide further information and illustrations of the pollen of *Takhtajania* based on liquid-preserved material. This will enable further comparisons to be made with the pollen of other genera of Winteraceae, which were studied in detail by Praglowski (1979). It should be noted that the material used in the present study was obtained about 150 km southeast of the original type collection. This raises the slight possibility that some differences between pollen in previous and present studies may be the result, not only of dried versus preserved material, but also of differences between the two populations of *Takhtajania*. For as Smith (1943), Vink (1970), Ehrendorfer et al. (1979), and others have demonstrated, there can be considerable morphological variability between populations of some species in the Winteraceae. However, such differences have not been demonstrated for their pollen grains. Generic names used follow those of Praglowski (1979), except that as recommended by Smith (1969) *Tasmannia* is substituted for *Drimys* sect. *Tasmannia*. The merging of *Bubbia*, *Belliolum*, and *Exospermum* into *Zygogynum* (Vink, 1985) is therefore not followed in the present paper.

[1] I thank Peter Raven and George Schatz for providing material of *Takhtajania perrieri* for this study. Special thanks are tendered to Barry Martin and Karen Reader for assisting with the electron microscopy. I wish to thank Dallas Mildenhall for assistance with acetolysis of the pollen and John Dawson and Ilse Breitwieser for translating papers in French and German, respectively. The School of Biological Sciences, Victoria University of Wellington, kindly provided financial assistance for the electron microscope studies.

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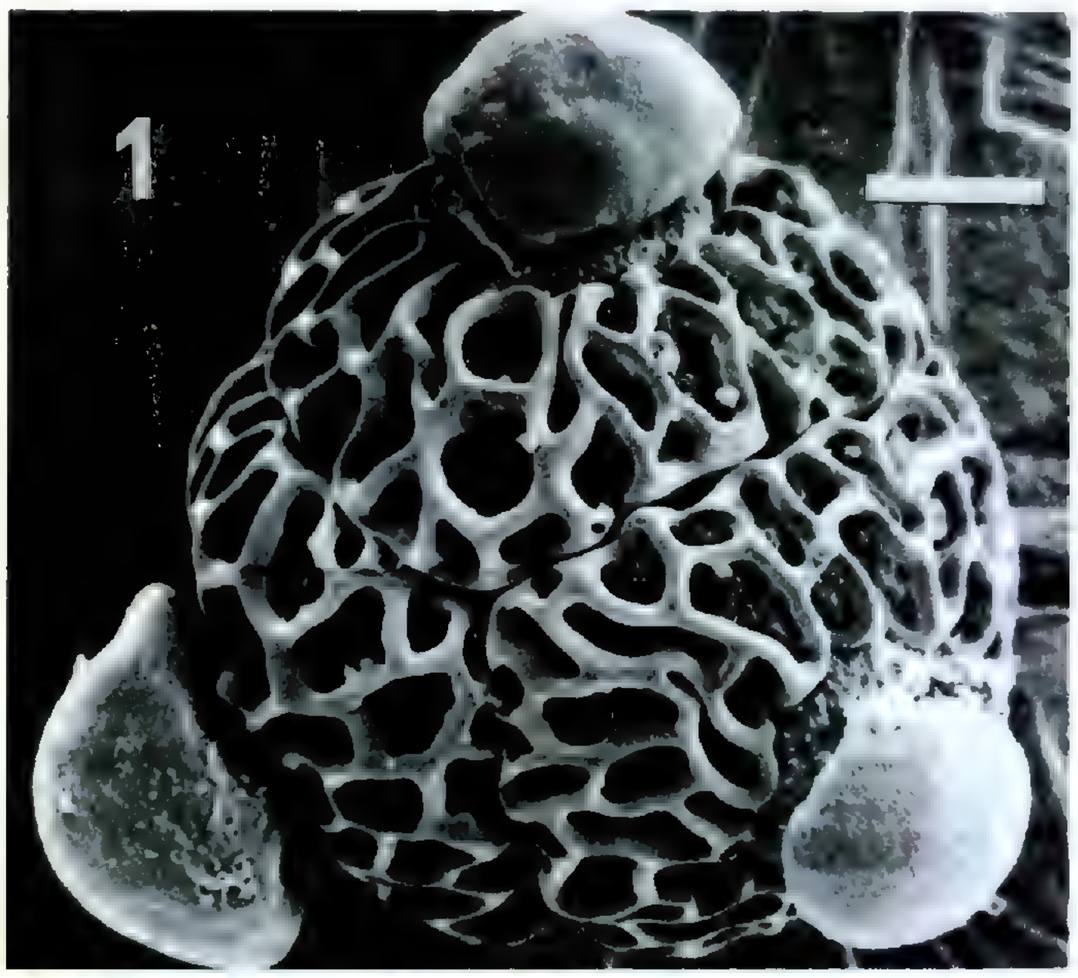
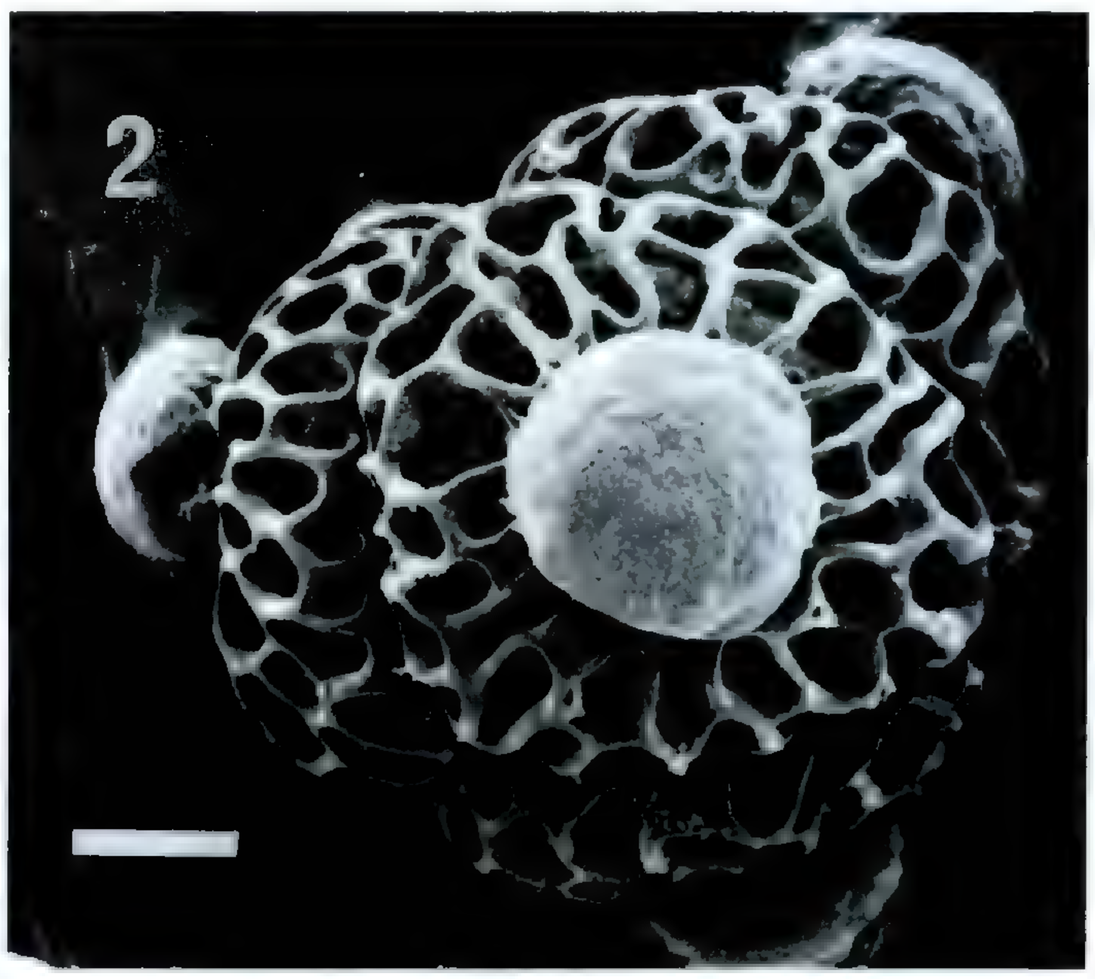
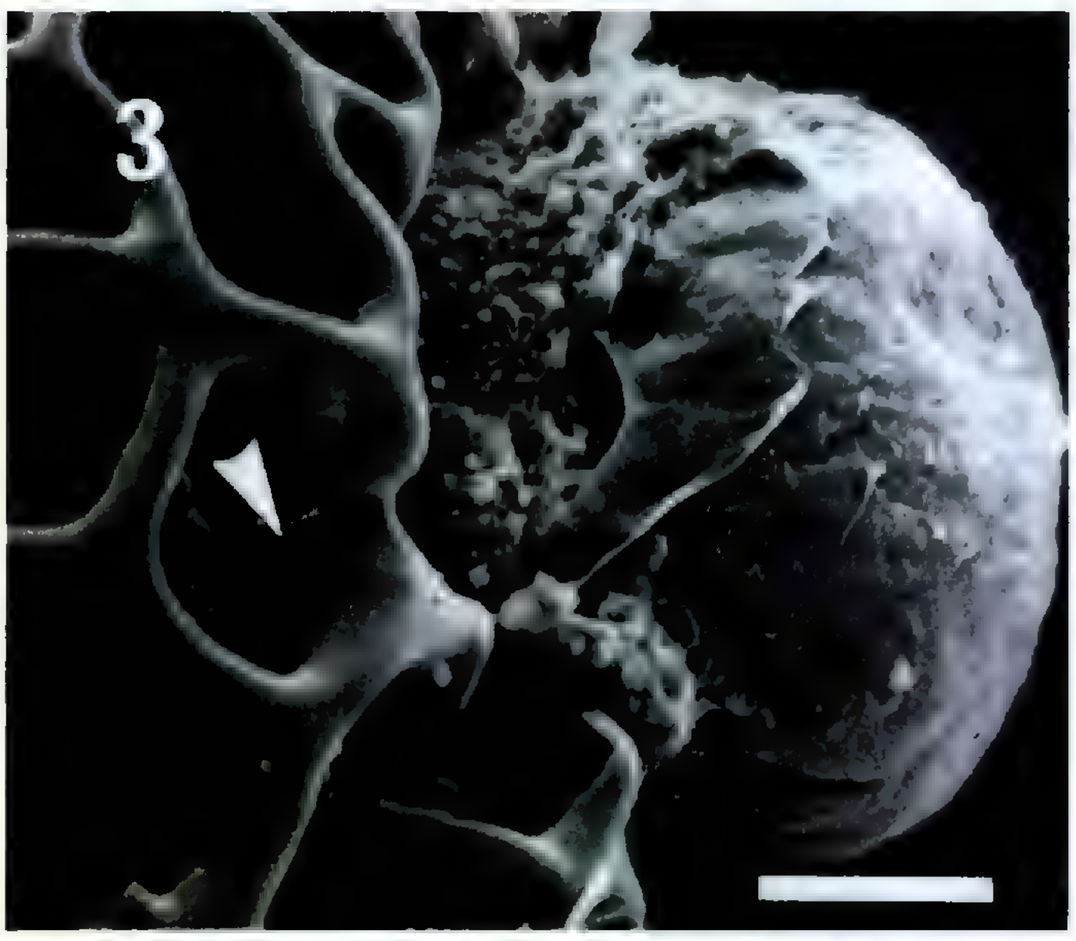
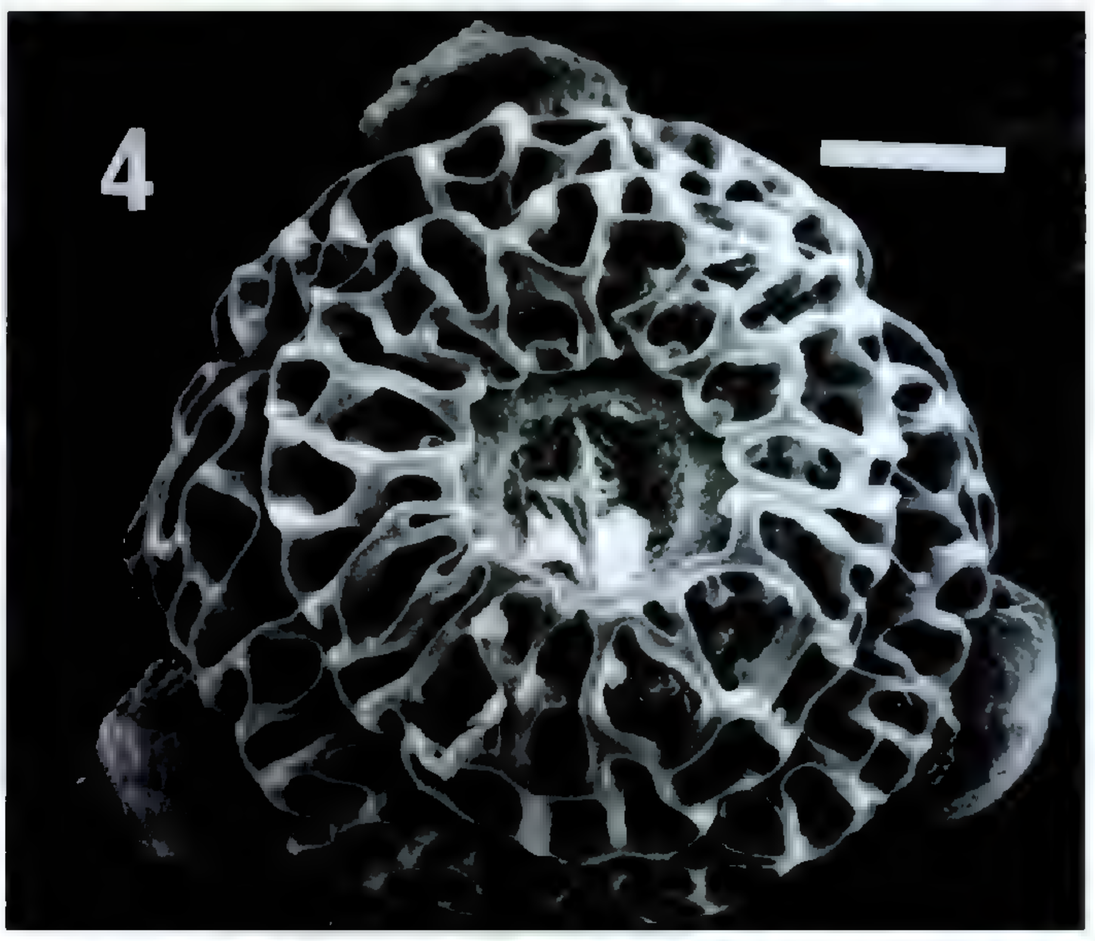
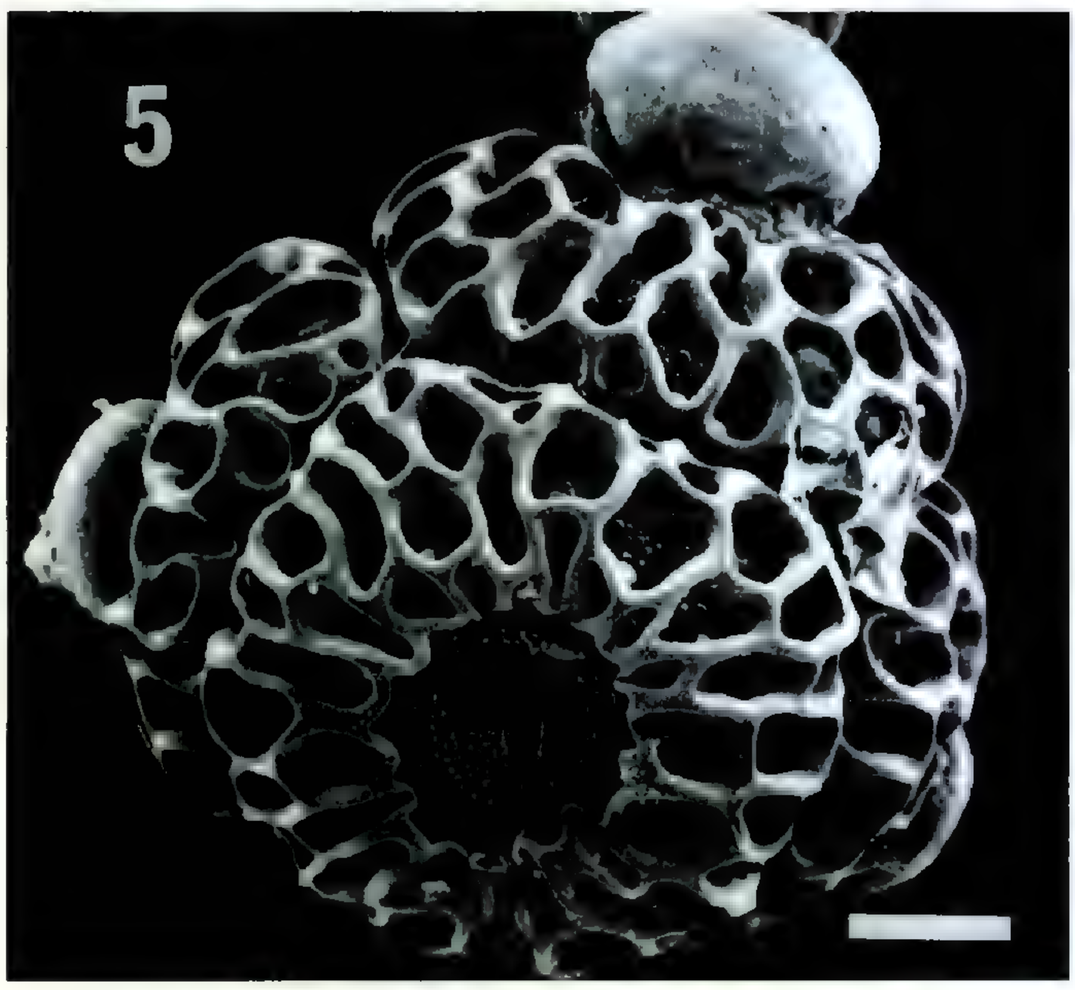
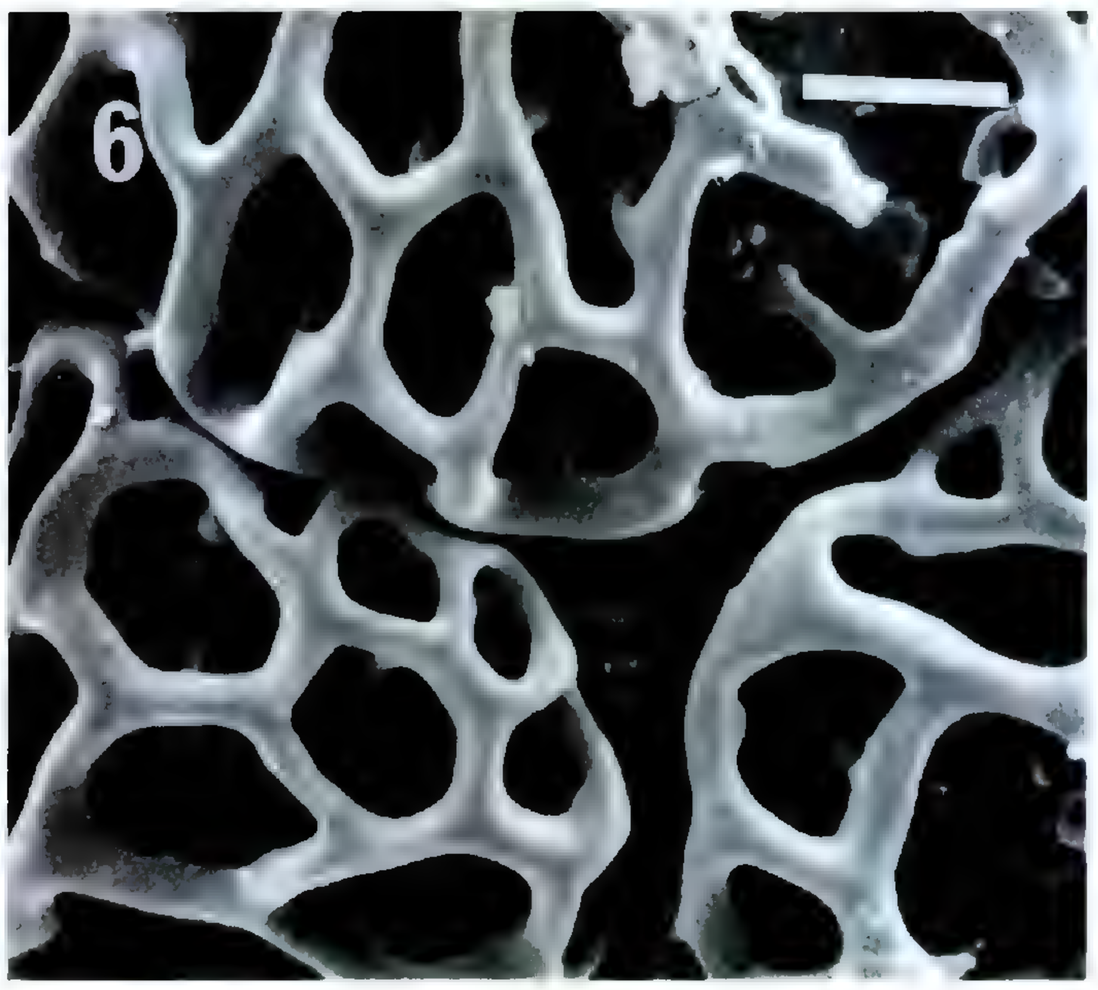




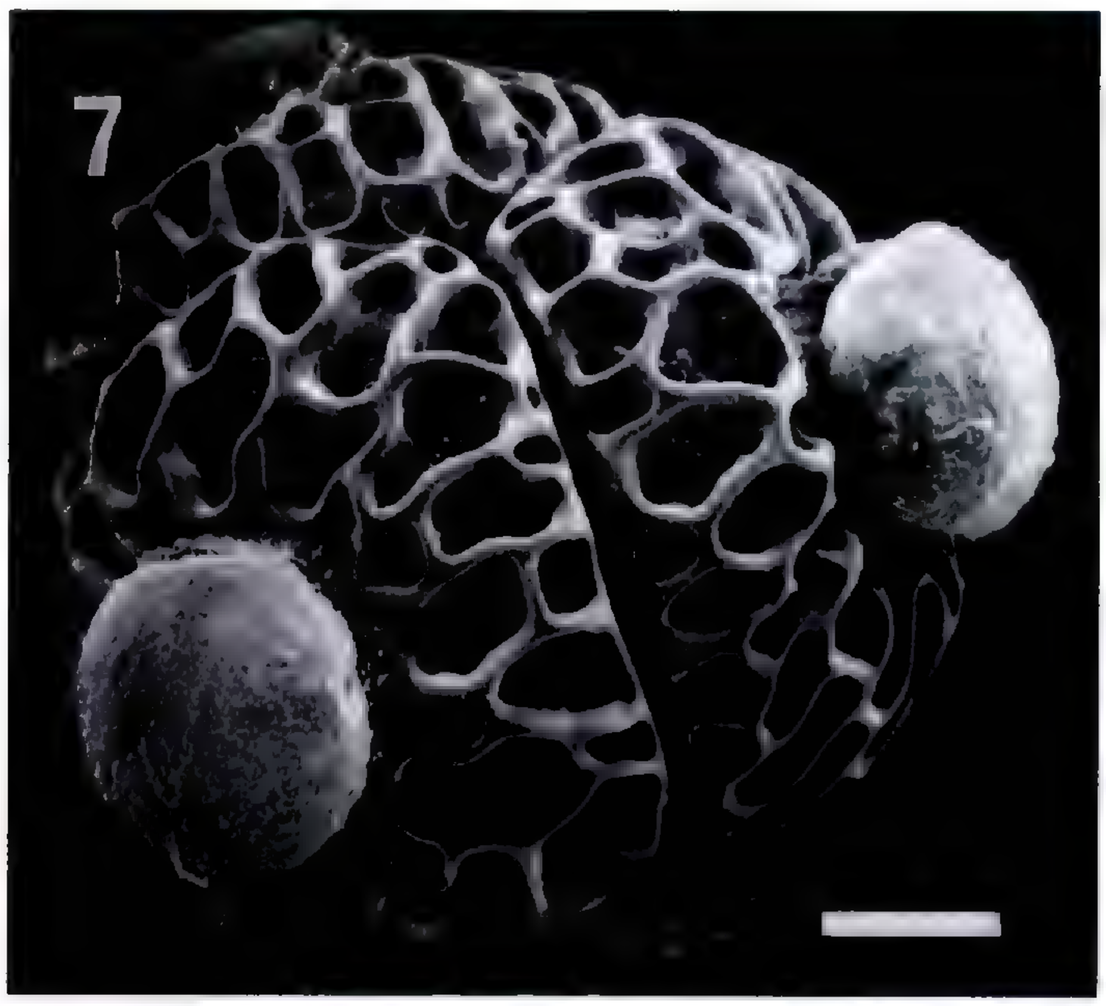


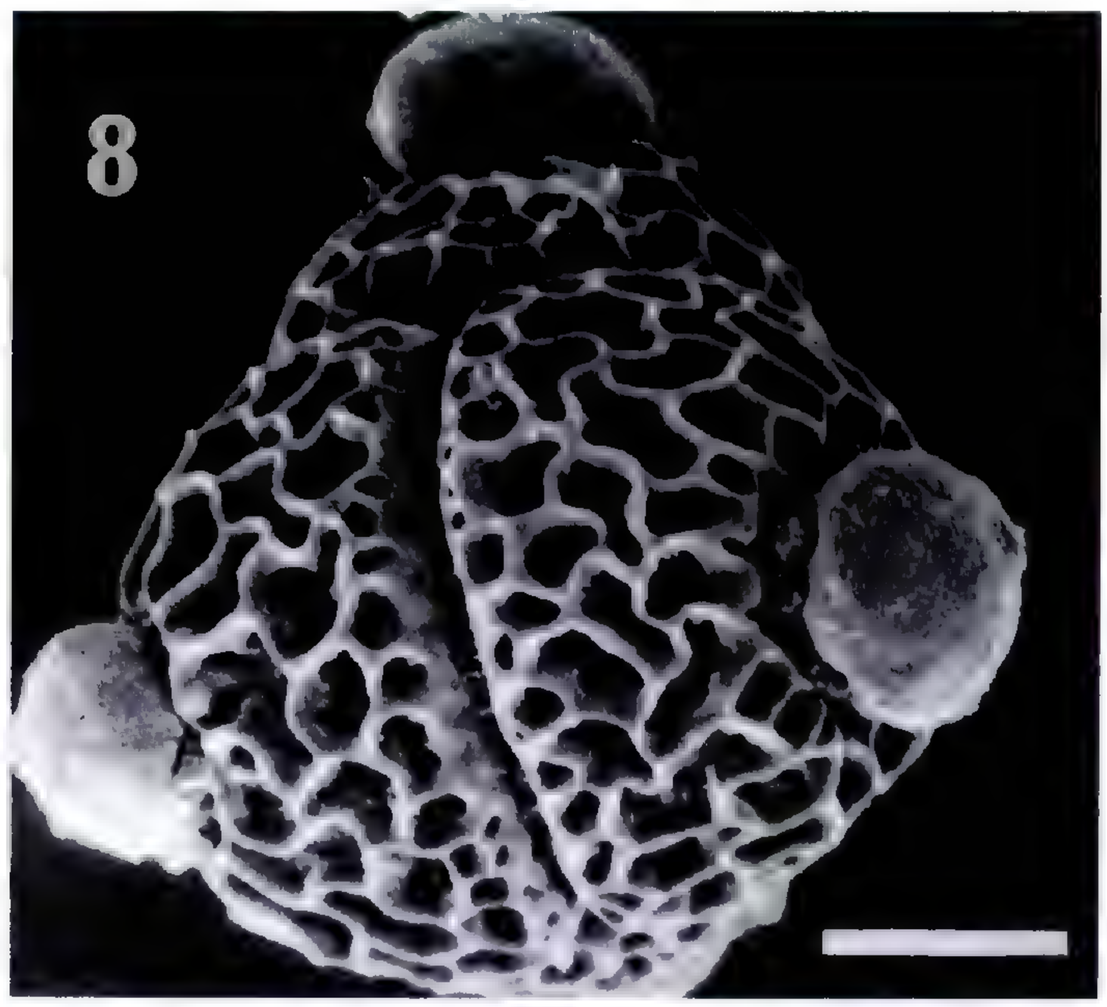


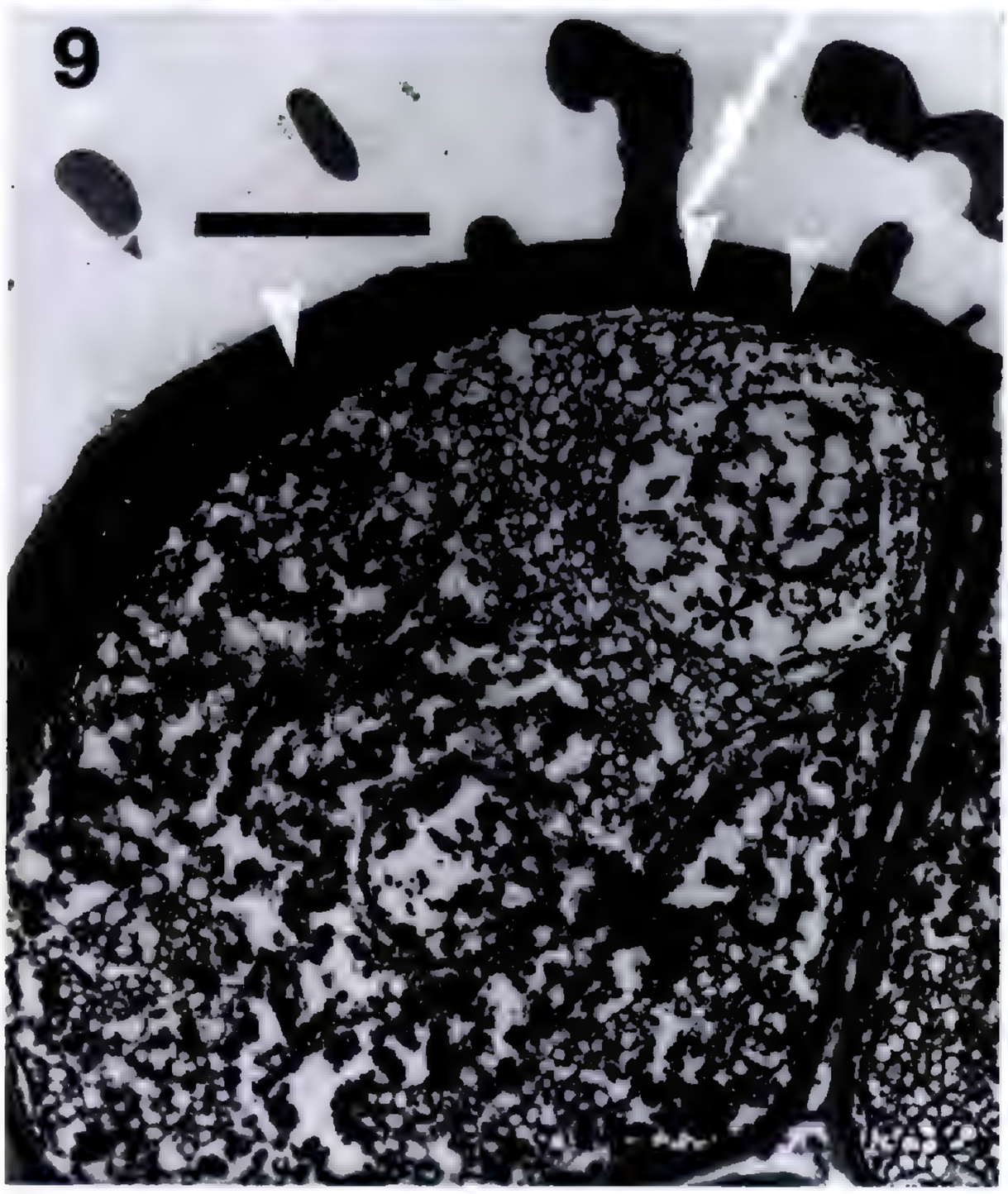


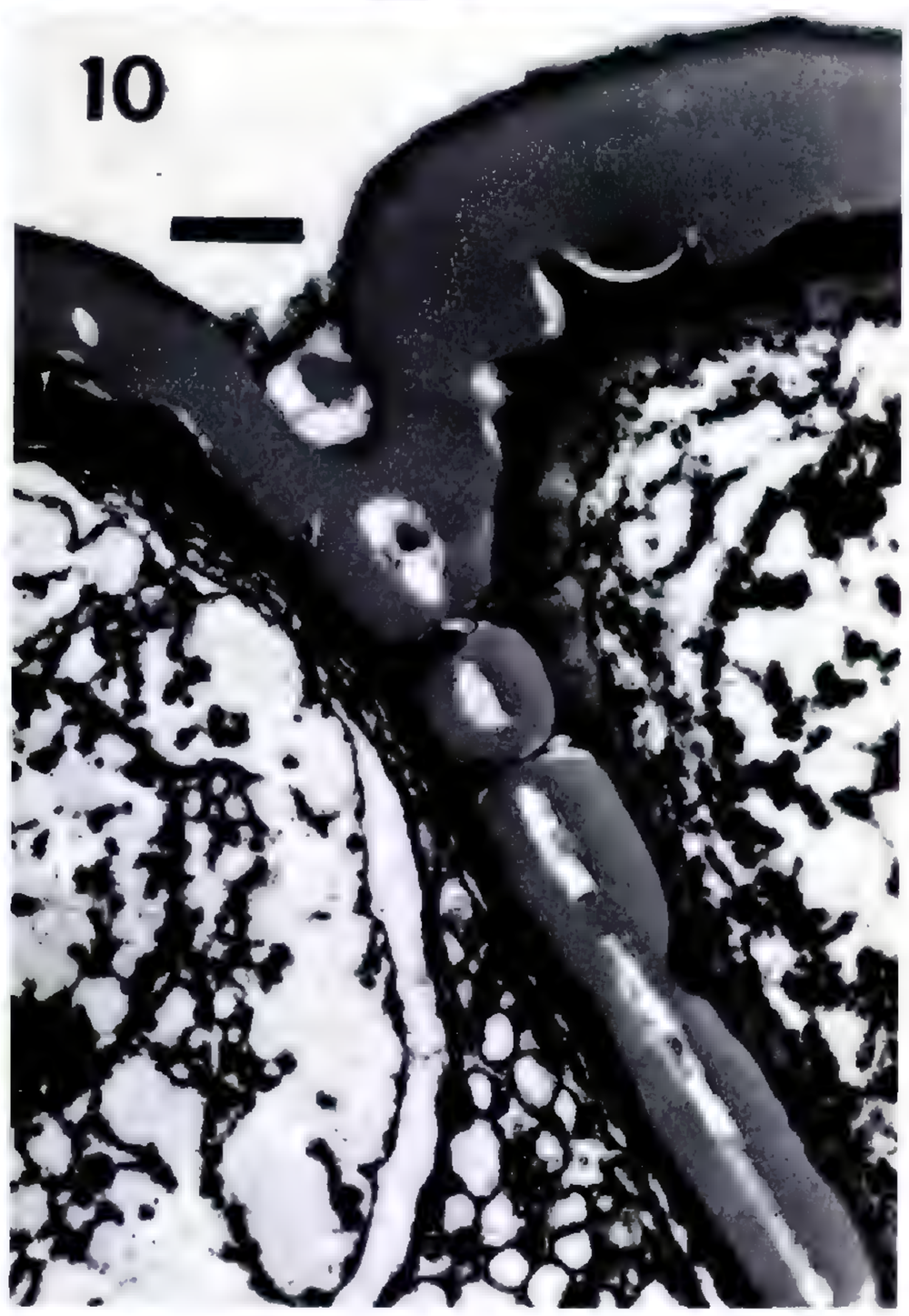


Figures 1–6. *Takhtajania perrieri* (SEM micrographs). —1. Pollen tetrad showing three of the four protuberant apertural papillae (scale bar = 10 μm). —2. Pollen tetrad with the uppermost pollen grain in polar view and all four apertural papillae visible (scale bar = 10 μm). —3. Apertural region of a pollen grain in lateral view. Annulus region indicated by asterisk; white arrow shows expanded base of a columella (scale bar = 5 μm). —4. Pollen tetrad with a sterile pollen grain uppermost, which lacks a protuberant papilla, with the annulus surrounding the aperture in polar view (scale bar = 10 μm). —5. Pollen tetrad with a sterile pollen grain (lowermost) in which the aperture has become torn into a colpus-like shape (scale bar = 10 μm). —6. Center of a pollen tetrad with portions of three pollen grains visible (scale bar = 5 μm).









Figures 7–10. —7. *Takhtajania perrieri* (SEM): a pollen tetrad with apertural regions of two pollen grains (lower left and upper right) in semi-lateral view (scale bar = 10 μm). —8. *Pseudowintera colorata* (SEM): a pollen tetrad with approximately similar orientation to the previous figure (scale bar = 10 μm). —9. *Takhtajania perrieri* (TEM): section of part of a tetrad showing most of a pollen grain in non-median section passing though the external fringes of the annulus, with white arrows indicating the endexine, a black arrow indicating the intine, and an asterisk showing the cytoplasm of the generative cell, which has a central nucleus (scale bar = 5 μm). —10. *Takhtajania perrieri* (TEM): section of portion of a tetrad illustrating part of the external and internal boundary between two grains. The arrows indicate gaps in the extexine, which have been filled with endexine (scale bar = 1 μm).

Material and Methods

Flowers and flower buds of *Takhtajania perrieri* (Capuron) Baranova & J.-F. Leroy were collected (*P. J. Rakotomalaza et al. 1342*) on 13 June 1997 from the Anjanaharibe-Sud Special Reserve, Antsiranana, Madagascar, and fixed in FAA.

Mature and nearly mature pollen for scanning electron microscope (SEM) study was dehydrated in an ethyl alcohol/acetone series, critical-point dried, sputter-coated with a thin layer of gold, and examined with a Philips 505 SEM. Pollen for transmission electron microscope (TEM) study was post-fixed in 1% OsO_4, dehydrated, embedded in Spurr's

resin, sectioned, and stained with uranyl acetate and lead citrate. Some mature pollen for light microscopy (LM) was acetolyzed and some was dehydrated in a TBA series, embedded in Paraplast, sectioned with a rotary microtome at 10 μm, and stained with haematoxylin, safranin, and fast green.

Measurements of external pollen features were based on LM using acetolyzed material to enable comparison with other genera of Winteraceae described in the pollen monograph of Praglowski (1979); 50 tetrads were sampled.

For comparative purposes, pollen tetrads of *Pseudowintera colorata* (Raoul) Dandy, obtained from native forest, Brown Cow Ridge, N.W. Nelson, New Zealand (voucher *WELTU 12949*), were examined under SEM, with treatment and fixation as for *Takhtajania*.

Results

External morphology

The most striking feature of the pollen tetrads of *Takhtajania*, which is shared by some other members of the family, is an expansion of the aperture, suggesting premature commencement of growth of the pollen tube (Figs. 1, 2). There is minor variation in the size of these "protuberant papillae" (Bailey & Nast, 1943) even between grains of the same tetrad, which may reflect differences in the degree of hydration of individual pollen grains. The surface of the aperture membrane is relatively smooth, in contrast to a surrounding verrucate (warty) annulus (Fig. 3). A few tetrads were observed in which one or more of the pollen grains did not show a bulging of the aperture (Fig. 4). Sections of tetrads revealed that such grains were sterile. Sometimes the aperture membranes of these pollen grains were torn (Fig. 5), with the ruptured part varying in shape.

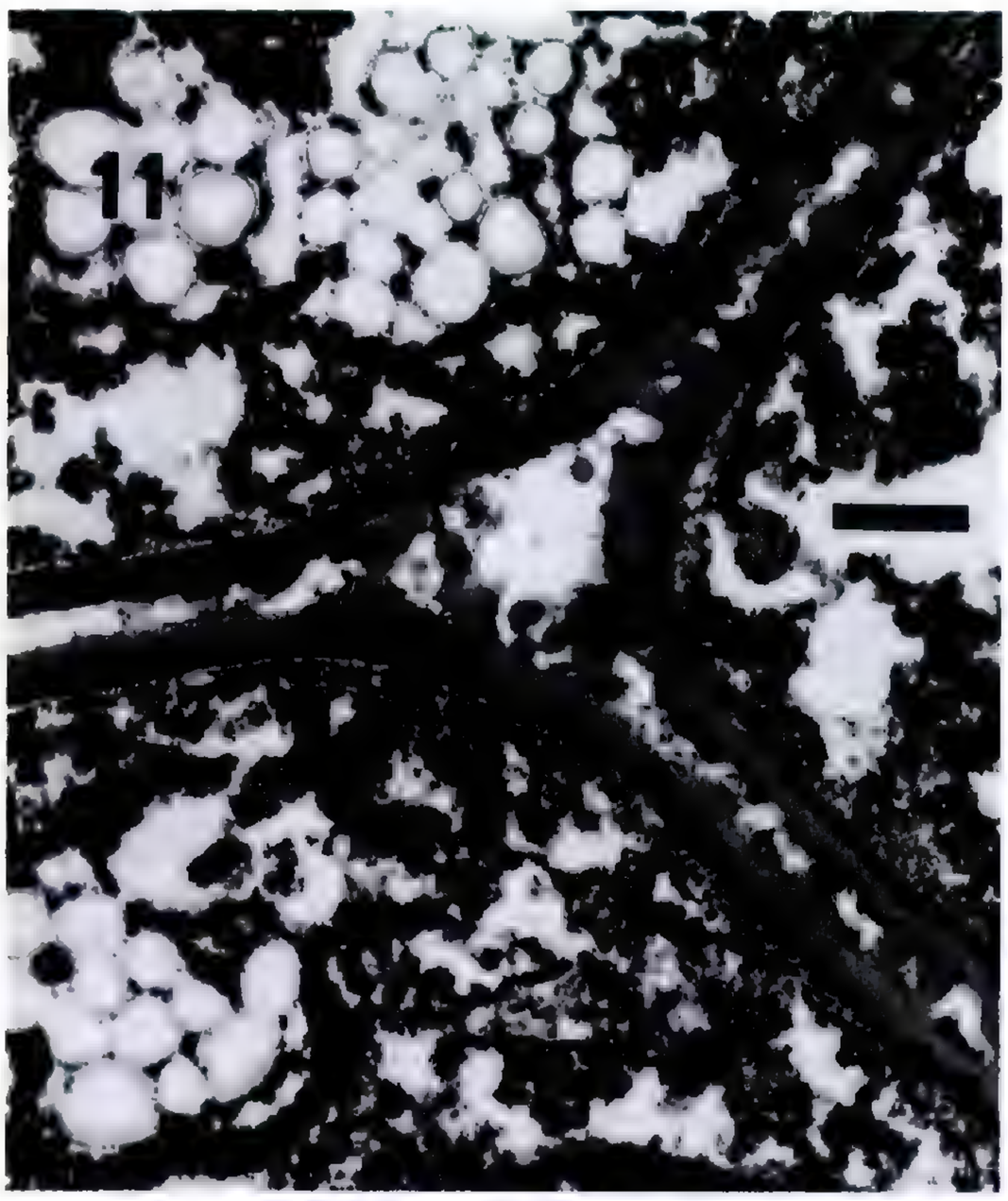
The tetrads are of the acalymmate type (Van Campo & Guinet, 1961), "in which each individual pollen grain is encompassed by its own ectexine which is usually incompletely developed in the septal part" (Praglowski, 1979: 4), as in other Winteraceae. Pollen grains are in tetrahedral tetrads, radially symmetrical, heteropolar, semitectate, and rounded to rounded-triangular in polar view (Fig. 2), uniaperturate with distal polar apertures, mostly round (ulcerate), although a few were oval. In lateral view, each pollen grain has a hemispherical distal (exposed) face (Fig. 1), excluding the shape of the apertural protrusion, and a proximal region, where the grain is joined to the other three members of the tetrad, consisting of three flattened inclined rather triangular surfaces converging near the "geometrical centre" of the tetrad. Praglowski (1979: 4) defined this as "the point at the centre of the free space between four coalescing pollen grains of a tetrahedral tetrad."

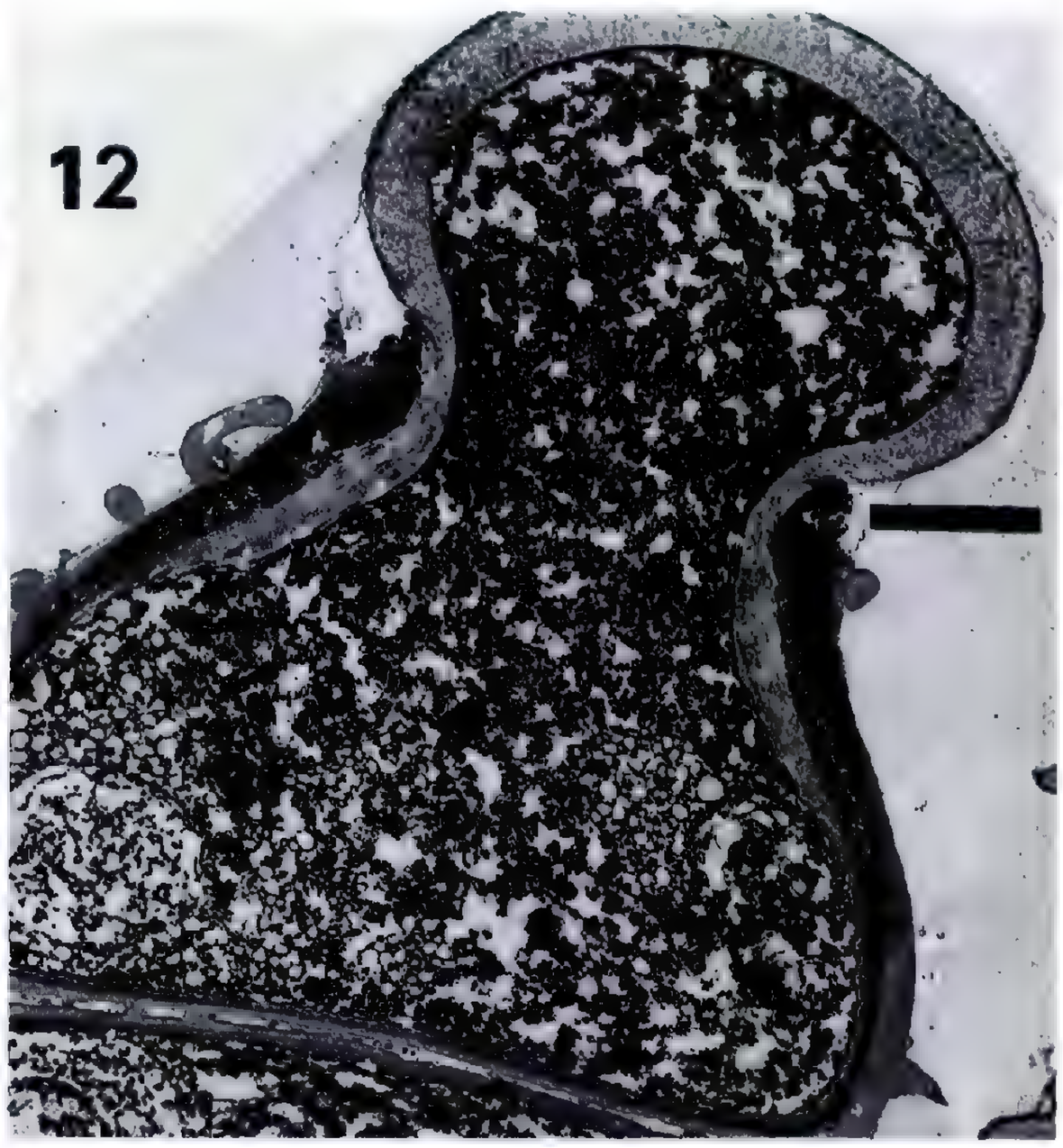
Diameter of tetrads, excluding apertural protrusions, which are destroyed by acetolysis, 65–83 μm (mean 74 μm); polar axis of individual pollen grains 23–33 μm (28 μm); largest diameter perpendicular to the polar axis 50–60 μm (55 μm). More than 90% of the apertures examined were almost exactly circular with a diameter (including the surrounding annulus) of 13–25 μm (20 μm); mean diameter of apertures, excluding annulus, 12.5 μm. The few oval apertures observed ranged from 6 × 10 μm to 8 × 11 μm. Width of annulus ca. 2.5–6 μm (Fig. 4).

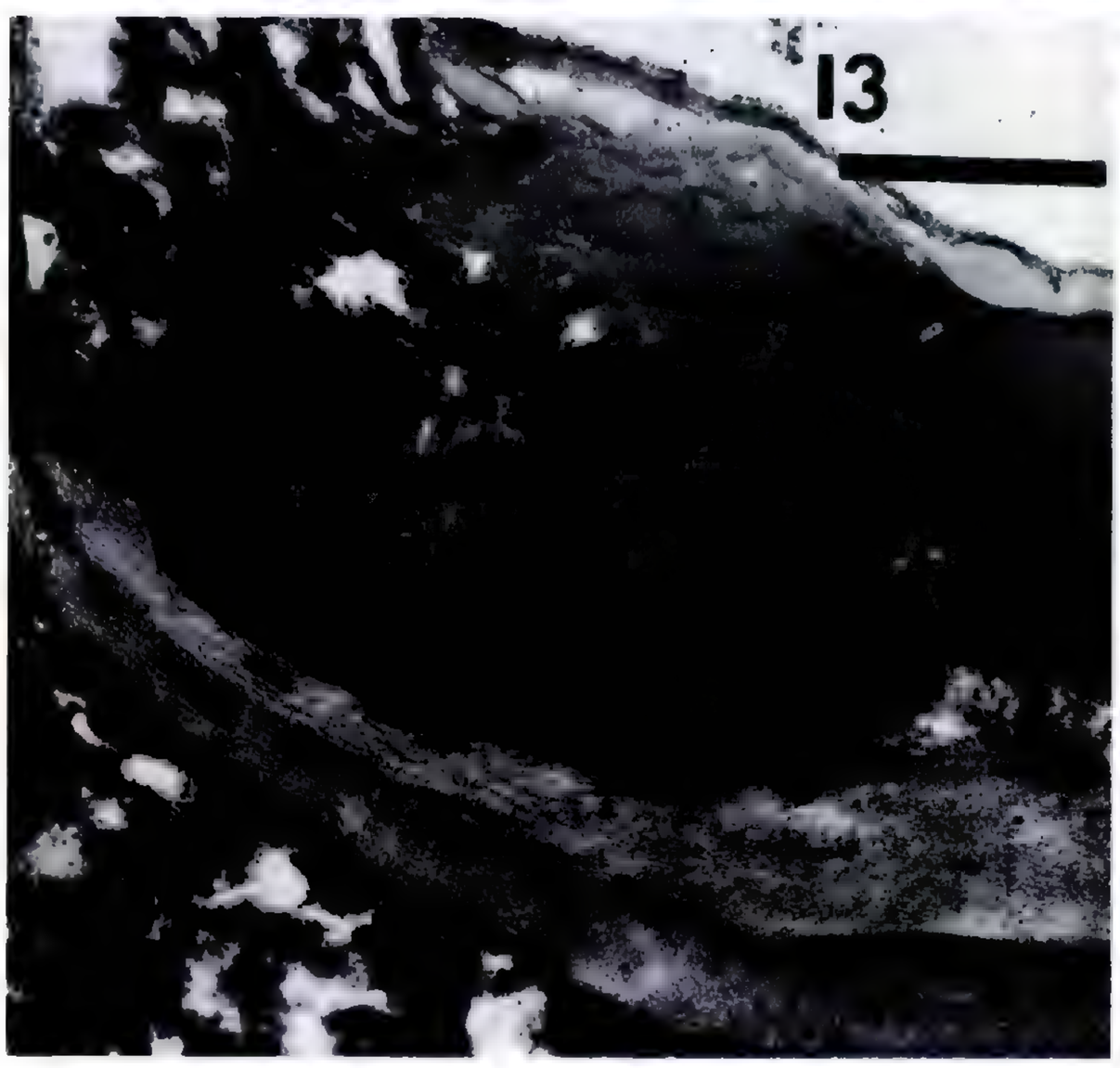
The reticulum on exposed surfaces of the tetrads has mostly medium-sized lumina with shapes ranging from square, rectangular, or polygonal to oval, circular, and irregularly curved sinuous outlines (Figs. 1, 2, 4–7). Muri forming the reticulum are, therefore, straight or curved and not invariably winding, as Praglowski (1979) found in pollen from herbarium material. As Praglowski (1979) noted, there are about 25 to 30 lumina in a pollen grain seen in lateral view (Fig. 1). Some lumina are smaller near the borders between members of the tetrad (Figs. 1, 6, 7) and, as in all members of the family with tetrad pollen, the reticulum (or perforate tectum in *Exospermum*) is completely interrupted near where individual grains are fused (Figs. 1, 6, 7). Only unbranched columellae were observed supporting the muri. Lobreau-Callen (1977) noted that rarely the columellae are duplicolumellate, although Praglowski (1979) commented this was not clearly confirmed. Columellae are cylindrical, not quite as wide as the muri, and they are usually splayed out at their proximal ends forming a rounded flattened cushion, which joins onto the foot layer (Figs. 3, 6). The outer surface of the foot layer is mostly smooth, but at intervals there are small, mostly spherical, ornamentations of varying size (Figs. 1–7). Thickness of the sexine was 4–6 μm and the nexine up to 1.5 μm, excluding thinner parts near the aperture of each pollen grain.

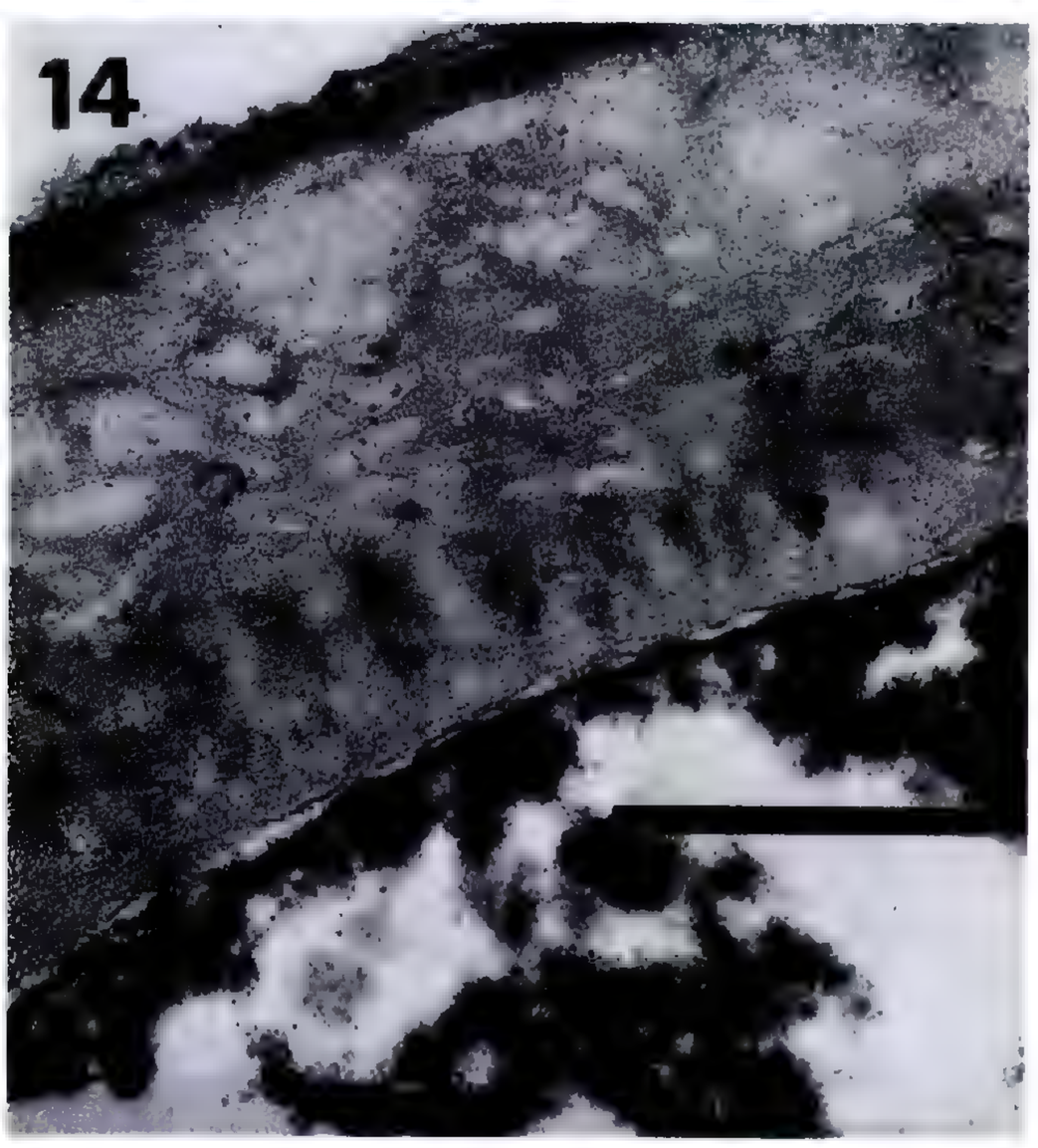
Ultrastructure

Extraseptal ectexine is 5–7.0 μm thick, excluding thinner regions near apertures. Muri are elliptical to obovate in cross section and generally about 1.5 μm wide and 1.2–2.2 μm high, with a height/width ratio of 1:1 to 3:2. Columellae ca. 2–4 μm in height, i.e., as high as or higher than muri. In extraseptal parts, the foot layer is continuous except in apertural regions of the tetrads and is thickest









Figures 11–14. *Takhtajania perrieri* (TEM micrographs). —11. Section through the center of a tetrad, with the interseptal zone of fusion forming a space near the center (scale bar = 1 μm). —12. Median section of most of a pollen grain in a tetrad passing through the center of an apertural papilla (scale bar = 5 μm). —13. Section through part of the annulus of a pollen grain with an asterisk indicating the center of the lamellated endexine (scale bar = 1 μm). —14. Section through part of the wall of an apertural papilla with underlying peripheral cytoplasm (scale bar = 1 μm).

near the exposed fringes of the pollen grains, where it is 0.5–1.0 μm thick and of similar thickness to the columellae and tectum (Fig. 9). It decreases in thickness toward the distal aperture of each pollen grain (Fig. 9). In septal parts (where the members of a tetrad are contiguous), the foot layer is thinner and measures ca. 0.1–0.4 μm. The inner surface of the foot layer is smooth to undulate (Figs. 9–11). Coherence between members of a tetrad is achieved by fusion of short columella-like elements extending from the foot layers of adjacent pollen grains (Figs. 9–11). In a few places there are small breaks in the foot layer in septal regions, which are "plugged" by endexine in mature tetrads (Fig. 10).

Beneath the foot layer is an endexine that is considerably thinner than the foot layer, except near the aperture (Figs. 9, 12). In septal regions, it is approximately one-third the thickness (0.1–0.2 μm) of the foot layer and even at low magnifications is readily distinguishable by its greater electron den-

sity (Figs. 9–11). In extraseptal regions, excluding the annulus, it is ca. 0.2–0.3 μm in thickness. It reaches a comparatively massive thickness of up to 3 μm in the annulus region, and here it is made up of compressed lamellar elements more or less tangentially aligned (Fig. 13). Near the external fringes of the annulus and somewhat beyond this, the endexine has an outer, more homogeneous part and an inner region consisting of irregular globules, presumably sporopolleninous, separated by electron-transparent spaces giving it a spongy appearance (Figs. 9, 12).

The intine, which underlies the endexine, is of similar thickness to the latter in septal regions (0.05–0.2 μm) and has a homogeneous appearance. It is usually readily distinguishable from the endexine, even at low magnifications (Fig. 9), because of its lower electron density. As Figure 12 illustrates, extraseptal intine increases dramatically in thickness toward the apertures, where it reaches up to 1.8 μm, and as commonly occurs in other Winteraceae (Praglowski, 1979) it loses its homogeneous appearance. There is a further increase in thickness of the intine in the apertural papilla region to up to 2.4 μm, where the wall appears to consist entirely of intine (Fig. 14). It can be seen that in these thicker regions, outer parts of the intine have inclusions of a vesicular-like nature, which is common in similar regions of many angiosperms. Only the innermost part of the intine appears more or less homogeneous (Fig. 14).

Discussion

It would seem that artifactual splits in the aperture in dried pollen, which had been obtained from the type specimen, similar to that shown in preserved pollen in Figure 5, led Straka (1963, 1975) to describe the pollen of *Takhtajania* as colpate and sometimes trichotomocolpate. Figure 1c in Straka (1963: 357) is a photomicrograph showing a triradiate tear in the aperture membrane, which is labeled as a "trichotomocolpate" aperture. Similarly, Lobreau-Callen (1977: 447) illustrated what was termed a colpate (slit-shaped) aperture in a photomicrograph (plate 1, fig. 4), which is very similar to the torn aperture shown in Figure 5 of the present paper. Praglowski (1979: 19) also noted apertures were different from other Winteraceae in their great variation in shape "ranging from pori to colpi." It is concluded, therefore, that previous reports of these varied types of apertures in *Takhtajania* are incorrect, and that it resembles other members of the family in possessing round or somewhat oval apertures. The study by Lobreau-Callen (1977) was the only previous one to illustrate pollen of *Takhtajania* under SEM, and two of his illustrations (plate 2, figs. 1 and 4) do show a bulging apertural membrane, despite the fact that pollen was obtained from dried herbarium material. It is unfortunate that it has become well established in the literature that *Takhtajania* pollen is sometimes trichotomocolpate (trichotomosulcate), but one can appreciate the limitations imposed on previous workers when studying pollen from a type specimen collected in 1909. On the other hand, true trichotomocolpate apertures are well defined. For example, the recently discovered genus *Anacostia*, a new basal angiosperm from the early Cretaceous of North America and Portugal, has some pollen grains with clearly delimited and attractively ornamented trichotomocolpate apertures (Friis et al., 1997).

The present investigation supports previous studies that *Takhtajania* has considerably larger tetrads than other members of the family whose pollen has been examined. Endress et al. (2000 this issue) suggested that the presence of these large tetrads may indicate polyploidy, as indicated for some other Winteraceae by Hotchkiss (1955). Straka (1963) stated that the acetolyzed pollen tetrads were 60 μm in diameter; Lobreau-Callen (1977) noted their mean diameter was 57.4 μm, without citing the range of sizes; and Praglowski (1979), who gave mean and size range for the pollen of most other Winteraceae examined, gave a single size of 65 μm diameter for the acetolyzed tetrads, presumably because of the limited material available. The range of tetrad size obtained in the current study (65–83 μm) extends the upper limits of the size range, but no tetrads were found as small as those measured by Straka (1963) and Lobreau-Callen (1977). The mean (74 μm) is considerably higher than that given by Lobreau-Callen (1977). It should be emphasized that acetolysis can increase the size of pollen units by 30% or more, and the extent of the increase may depend on methods of acetolysis used (D. C. Mildenhall, pers. comm. 1999). In fact, tetrads of *Takhtajania*, which had been dried by the critical point method for SEM (Figs. 1, 2, 4, 5, 7) had a diameter (excluding the apertural papillae) averaging ca. 47 μm. It is highly probable that the tetrads of other Winteraceae undergo a similar increase in size with acetolysis. Thus, *Pseudowintera colorata*, which had tetrads with a mean diameter of 50 μm following acetolysis (Praglowski, 1979), measured ca. 35 μm under SEM, when material has been dried by the critical point method (unpublished pers. obs.).

Lobreau-Callen (1977) and Praglowski (1979)

made detailed comparisons between the pollen of *Takhtajania* and other genera in the family. Lobreau-Callen (1977) concluded that based on its structure and sculpture, its pollen is closest to that of *Belliolum,* from which *Takhtajania* can be distinguished by the difference in size of perforations in the internal walls (septae), which are large in *Belliolum.* It was subsequently found for several genera in the family that if these gaps are still open at the time of pollen grain mitosis and therefore the four members of a tetrad share a common cytoplasm, division into tube and generative cells within each pollen grain is synchronous within a tetrad, e.g., in *Belliolum* (Sampson, 1981). It is probable that pollen grain mitosis is asynchronous within each tetrad of *Takhtajania,* because the small and infrequent gaps in the ectexine forming the septae are plugged with endexine, which is formed before division—at least in those species that have been investigated. On the other hand, species with synchronous division have, in older tetrads, the septal gaps plugged with intine, which is formed subsequent to pollen mitosis (Sampson, 1981), or large gaps may remain open in mature tetrads, as illustrated by Praglowski (1979) for *Bubbia howeana* (F. Muell.) Tiegh. Assuming that *Takhtajania* has asynchronous pollen mitosis, it shares this feature with *Pseudowintera* and *Drimys.* Whether or not this indicates a relationship between these three genera depends on whether asynchronous mitosis, the adaptive significance of which is not obvious, has evolved independently in these taxa. Furthermore, this feature has not been investigated in all species in the family, and future studies may demonstrate that it is not consistent within a genus. For example, although asynchronous division occurs in all three species of *Pseudowintera,* only one species of *Drimys* has been examined (Sampson, 1981). It has been suggested that the asynchronous type has evolved from the synchronous (Sampson, 1981).

In contrast to other Winteraceae, *Drimys, Takhtajania,* and *Pseudowintera* share protuberant apertural papillae, which may or may not indicate a relationship between the genera, depending on whether or not this characteristic evolved independently in these three genera. Bailey and Nast (1943) noted that van Tieghem (1900), who studied pollen of all genera (excluding *Takhtajania*), although he provided no illustrations, found that the pollen of the three New World species of *Drimys* he examined formed protuberant papillae when moistened, in contrast to *Tasmannia* (*Drimys* sect. *Tasmannia*), *Pseudowintera, Bubbia, Belliolum, Exospermum,* and *Zygogynum.* Bailey and Nast (1943) confirmed these results. However, fresh and preserved pollen of all three species of *Pseudowintera* have the papillae (Fig. 8). Both *Takhtajania* and *Pseudowintera* possess an annulus that is verrucate (Figs. 3, 8) with smaller verrucae in *Takhtajania,* in contrast to the comparatively smooth-surfaced annuli illustrated for species of *Drimys* (sensu Smith, 1969) by Lobreau-Callen (1977) and Praglowski (1979). Tetrads of *Pseudowintera* differ from those of *Takhtajania* in generally lacking expanded tips to their columellae where they touch the foot layer and in possessing numerous small perforations in the tectum near their external boundaries (Fig. 8).

Praglowski (1979), while agreeing that *Takhtajania* pollen differs considerably from that of *Bubbia,* the genus in which *Takhtajania perrieri* had first been placed (Capuron, 1963), disagreed with the conclusion of Lobreau-Callen (1977) that it was closest to pollen of *Belliolum.* Praglowski (1979) questioned the value of the ratio between the width of muri and the diameter of bacula (columellae) used by Lobreau-Callen (1977) for intergeneric segregation and concluded its pollen was closest to that of *Drimys* (syn. *Drimys* sect. *Drimys*). Lobreau-Callen (1977) also had noted similarities between *Takhtajania* and *Drimys* pollen. Coetzee and Praglowski (1988) described two types of fossil Winteraceae pollen resembling *Tasmannia* and *Bubbia* from the Miocene of South Africa. This discovery and the Early Cretaceous occurrence of Winteraceae pollen in Israel (Walker et al., 1983) led them to suggest that the Winteraceae had a West Gondwanan origin and an early differentiation, possibly in Africa, before migration to Australasia and Madagascar. They suggested furthermore that a dispersal route from the south could probably explain the close resemblance between the pollen of *Takhtajania* and *Drimys* of the New World. Doyle et al. (1990a) noted that extinct relatives of the Winteraceae (*Afropollis*) were an important component of Early Cretaceous tropical floras and extended into Laurasia, and commented that their present austral temperate distribution was attained later. The winteraceous affinity of *Afropollis* has, however, been questioned by Friis et al. (1999).

Ultrastructural detail in pollen of the Winteraceae (e.g., intine and endexine structure, endexine fine structure) does not seem to differ significantly between genera (Praglowski, 1979, and unpublished pers. obs.). However, further studies are needed to confirm this, utilizing freshly collected pollen and TEM fixation techniques.

Table 1, which includes information from Praglowski (1979), the present study, and unpublished personal observations, summarizes a number of pol-

Table 1. Some pollen features of Winteraceae. Abbreviations: T = tetrad, M = monad, * = includes *Zygogynum acsmithii* with tetrad pollen, N.A. = not applicable

Genus	*Takhtajania*	*Pseudowintera*	*Drimys*	*Belliolum*	*Bubbia*	*Tasmannia*	*Zygyogynum*	*Exospermum*
Tetrad diameter (μm)	65–83	36–55	34–55	34–48	33–50	32–39	32–51	37–43
Are tetrads circular in outline?	No	No	No	No	No	No	No	Yes
Are apertures surrounded by an annulus-like region?	Yes	Yes	Yes	Yes	Yes	No	No, T Yes, M	No
Do apertures have protuberant papillae?	Yes	Yes	Yes	No	No	No	No	No
Exine structure	reticulate	reticulate	reticulate	reticulate	reticulate	reticulate	reticulate, T tectate-perforate to micro-reticulate, M*	tectate-perforate to micro-reticulate
Number of lumina (1 grain-lateral view)	25–30	13–20	13–26	8–20	10–20	10–20	40–100	>100
Columellae shorter than thickness of the tectum?	No	No	No	Yes	No	Yes	No	No
Height/width ratio of muri	1:1–3:2	3:1	2:1	4:1	2:1 or 3:2	3:2 or 2:1	tetrads 3:2 monads, N.A.	N.A.
Pollen mitosis synchronous /tetrad	No(?)	No	No	Yes	Yes	Yes	Yes	Yes
Columellae appear two-layered because of thicker distal segments?	No	No	No	No	No	No	No	Yes
Maximum thickness of sexine (μm)	6.0	2.5–4.0	2.5–5.0	2.0–4.5	2.5–3.5	2.0–3.0	2.0–4.0, T 1.5–2.5, M	3.0
Maximum thickness of nexine (μm)	1.5	1.5	1.0	1.0	1.0	0.5	1.0, T 0.5–2.0, M	0.5

len characters of the genera. Assuming that some or all similar characters are not the result of convergent evolution, the pollen of *Takhtajania* most closely resembles that of *Pseudowintera* and *Drimys*. Endress et al. (2000) reached somewhat different conclusions when considering the total evidence of floral features and considered that *Takhtajania* fits best in the *Pseudowintera–Zygogynum* (including *Bubbia*, *Belliolum*, and *Exospermum*) clade, "which is sister to the *Tasmannia/Drimys* clade." Hopefully, further studies, including those using molecular data, will provide further information on the relationships between *Takhtajania* and other Winteraceae.

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EMBRYOLOGY OF *TAKHTAJANIA* (WINTERACEAE) AND A SUMMARY STATEMENT OF EMBRYOLOGICAL FEATURES FOR THE FAMILY[1]

Hiroshi Tobe[2] *and Bruce Sampson*[3]

ABSTRACT

We present the first report on the embryology of *Takhtajania*. By adding its data to those already known from other Winteraceae (*Drimys*, *Pseudowintera*, *Tasmannia*, and *Zygogynum* s.l.), we summarize embryological features of the family to evaluate the proposed basal position of *Takhtajania* in the family and a possible sister-group relationship between Winteraceae and Canellaceae, which were recently suggested by molecular evidence. Comparisons within and between the families showed that *Takhtajania* agrees well with other Winteraceae embryologically, having no distinct features to support its basal position in the family. Although Winteraceae and Canellaceae share a number of basic embryological features, including an exotestal seed coat, Winteraceae are clearly distinct by virtue of the following features: the outermost one of the middle layers and even part of the connective tissue in the anther developing fibrous thickenings at anthesis; ovules anatropous (not campylotropous as in Canellaceae); the micropyle formed by the inner integument alone (rather than by two integuments as in Canellaceae); an exostome formed after fertilization; a persistent micropylar part of the tegmen composed of variously enlarged, thick-walled cells; and the exotesta palisadal. Winteraceae are thus a well-defined group embryologically and, despite their modern widespread distribution, genera show little diversification in embryological characters.

Key words: Canellaceae, embryology, systematic anatomy, *Takhtajania*, Winteraceae.

The Winteraceae are a relatively small woody dicotyledonous family, comprising about 65 species assigned to *Drimys*, *Pseudowintera*, *Takhtajania*, *Tasmannia*, and *Zygogynum* sensu lato (including *Belliolum*, *Bubbia*, and *Exospermum*). They are broadly distributed from the Philippines to Tasmania and New Zealand, South and Central America, and Madagascar (Vink, 1993). Because of its relict Gondwanan distribution as well as its distinctive primitive-type floral morphologies, the family has long attracted the attention of researchers of plant evolution and morphology. Within the family, *Takhtajania*, comprising the only extant species *T. perrieri* (Capuron) Baranova & J.-F. Leroy in the Africa/Madagascar region, has been considered most isolated, and in fact it differs from the three other genera in having flowers with two carpels united to a paracarpous gynoecium, instead of free carpels or a number of carpels united to a eusyncarpous gynoecium. *Takhtajania* thus has been placed in a subfamily of its own, Takhtajanioideae, and the other genera in another subfamily Winteroideae (Leroy, 1978; Takhtajan, 1997).

Takhtajania has been very poorly understood in general with respect to its morphological and evolutionary traits because only a single collection made in 1909 had been available for research until recent times (Leroy, 1980). A number of trees were, however, rediscovered at the Anjahanaribe-Sud Special Reserve (14°45′S, 49°29′E) in Madagascar in 1994 (see Schatz et al., 1998; Schatz, 2000 this issue). An extensive collection was subsequently made for morphological, anatomical, cytological, and molecular studies (for many results, see other articles in this *Annals* issue).

In this paper we present the embryology of *Takhtajania*, which has not been studied before. Taking the data from this study together with those from the four other genera (*Drimys*, *Pseudowintera*, *Tasmannia*, and *Zygogynum* s.l.), which have already been studied relatively well, we will summarize embryological features of the whole family Winteraceae. Molecular evidence based on combined sequence data from mitochondrial *atp*1 and *mat*R, plastid *atp*B and *rbc*L, and nuclear 18S rDNA has revealed that Winteraceae are sister to Canellaceae

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with support of 100% bootstrap value, and that *Takhtajania* has a basal position within the Winteraceae (Qiu et al., 1999). We will discuss whether or not the sister-group relationship between Winteraceae and Canellaceae is supported, whether or not the basal position of *Takhtajania* in Winteraceae is supported, and how Winteraceae are a coherent group distinct from the Canellaceae.

Materials and Methods

Flower buds and fruits of *Takhtajania perrieri* in various stages of development were collected at the Anjahanaribe-Sud Special Reserve in Madagascar (vouchers: *Rakotomalaza et al. 1342, Schatz 3748, Ravelonarivo s.n.* in 1998; all at MO) and fixed with FAA (five parts stock formalin; five parts glacial acetic acid; 90 parts 50% ethanol). Twenty-eight flower buds, four open flowers, and 17 fruits were dehydrated through a *t*-butyl alcohol series, embedded in Paraplast (melting point 57–58°C), and sectioned using a rotary microtome following standard paraffin methods. Sections cut at about 6–10 μm thickness were stained with Heidenhain's haematoxylin, Safranin-O, and FastGreen FCF, and mounted with Entellan. To examine their fine structure, several anthers were embedded in Spurr's resin after dehydration through an acetone series and sectioned with glass knives using an ultramicrotome. Sections cut at about 1–2 μm thickness were stained with Toluidine blue O.

Observations

Anthers and Microspores

The small anther is supported by a more or less laminar filament (Fig. 1), and is tetrasporangiate (Figs. 2, 3). Prior to maturation the anther comprises 5 to 6 cell-layers: an epidermis, an endothecium, 2 to 3 middle layers, and a tapetum (Fig. 7). Because of the lack of younger flower buds, it is not certain how the anther wall developed. The tapetum is glandular, and its cells are 2-nucleate (Fig. 4). During maturation, the epidermal cells are enlarged to some extent but nearly collapsed eventually, and the endothecium develops fibrous thickenings (Figs. 8, 9). The middle layers mostly degenerate, but like the endothecium, the outermost layer usually develops fibrous thickenings (Figs. 8, 9). Part of the connective tissue may also develop fibrous thickenings (Fig. 8). Anther dehiscence takes place by longitudinal slits, with each slit common to two microsporangia of a theca (Fig. 9).

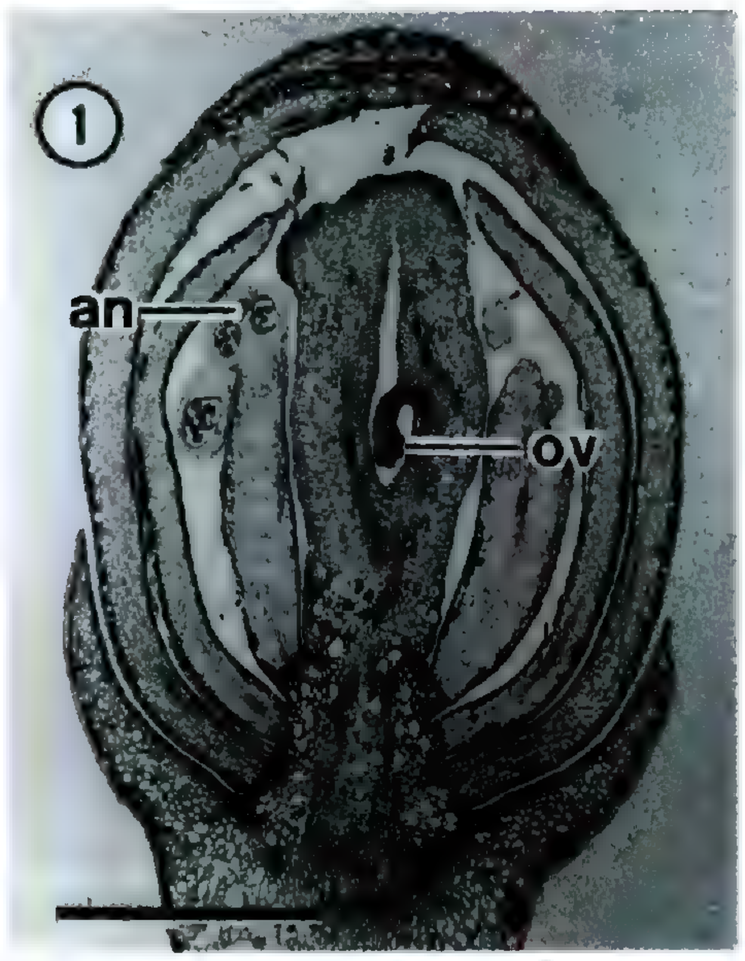
Meiosis in the microspore mother cell is accompanied by simultaneous cytokinesis (Fig. 5). The shape of resultant tetrads is tetrahedral. Unfortunately, no buds could be sectioned that would have showed stages in the division of the 1-celled microspores leading to the formation of vegetative (tube) and generative cells. Two different types of division have been found. In the synchronous type, which occurs in *Tasmannia* and *Zygogynum* s.l., the nuclei of all four pollen grains within a tetrad are at exactly the same stage of mitotic division because there are cytoplasmic connections through gaps in their internal walls. However, in the asynchronous type, which occurs in *Drimys* and *Pseudowintera,* division of the microspore nuclei is asynchronous because there are no gaps in the internal walls of each tetrad at this time (Sampson, 1981; Prakash et al., 1992). It has been suggested that the asynchronous type has evolved from the synchronous, and in view of the basal position of *Takhtajania* it would therefore be of interest to know if it too has the synchronous type of pollen mitosis.

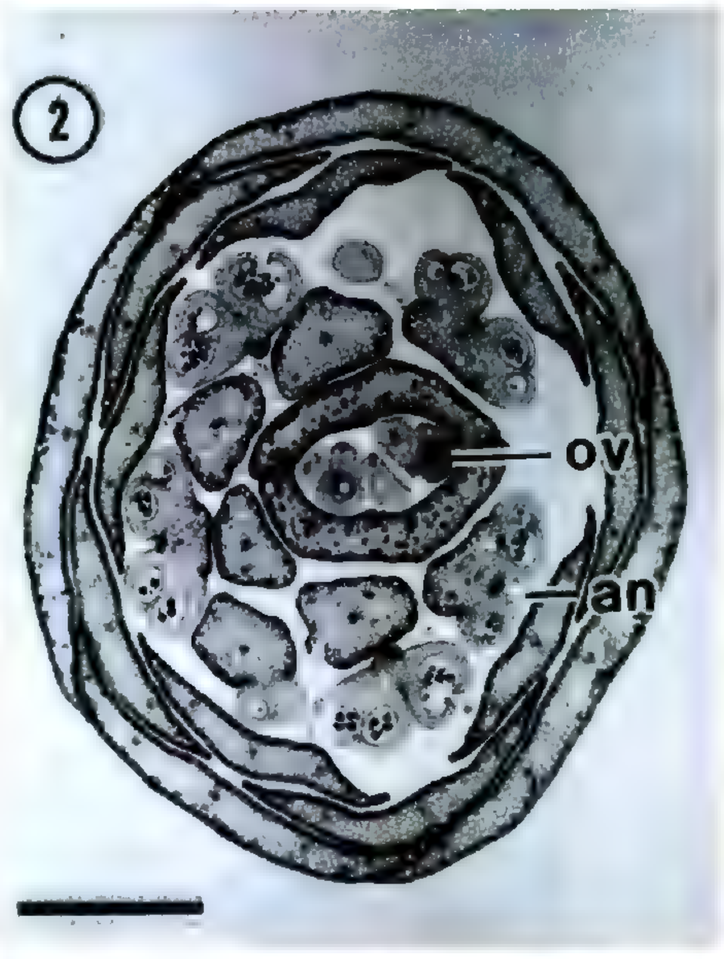
Pollen grains are 2-celled at the time of shedding (Fig. 6).

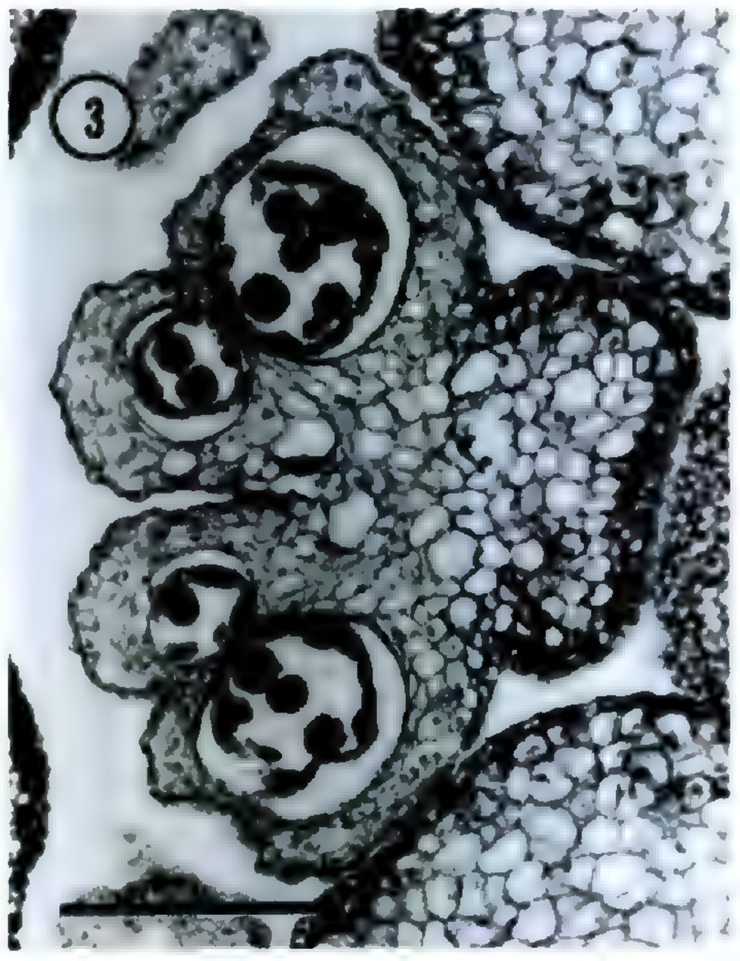
Ovules, Nucellus, and Megagametophyte

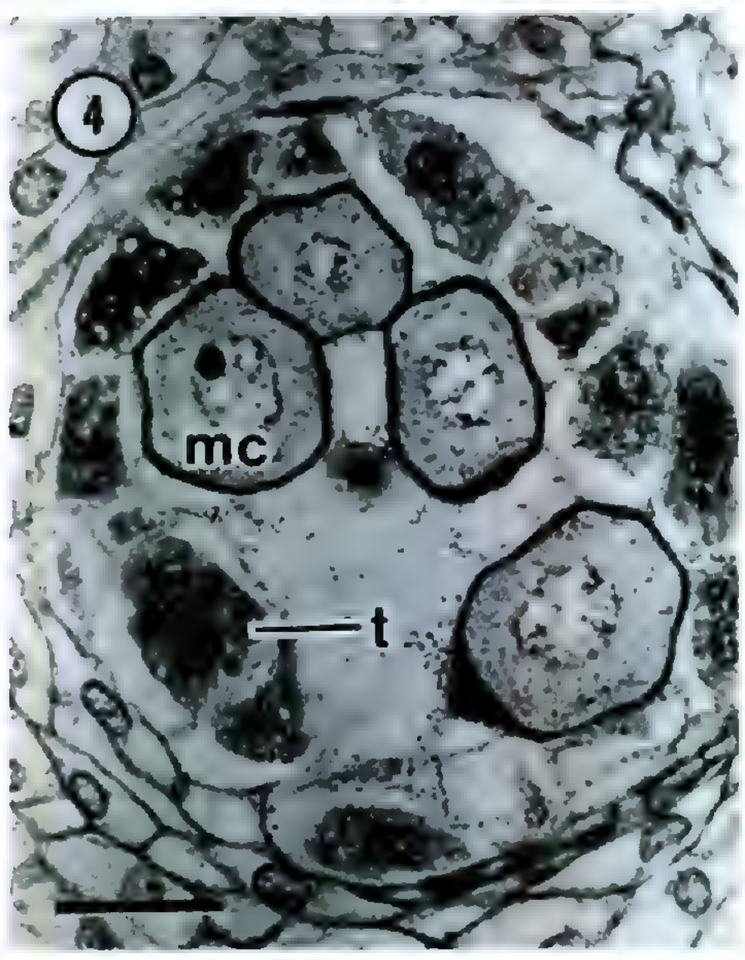
Early in development six to seven ovules with a long funicle are pendulous in an ovarian locule (Figs. 1, 2; see also Endress et al., 2000 this issue). They are anatropous at maturity (Fig. 15) and crassinucellate. The archesporium is 1-celled (Fig. 10). The archesporial cell divides periclinally to form the primary parietal cell above and the primary sporogenous cell below (Fig. 11). While the primary parietal cell further divides periclinally into more cells, the primary sporogenous cell develops into a megaspore mother cell. Thus ovules have one enlarged megaspore mother cell below a longitudinal row of five to six parietal cells. The megaspore mother cell undergoes meiosis to produce a linear tetrad of megaspores (Fig. 12). In the megaspore tetrad the chalazal megaspore functions (Fig. 12) and develops successively into a 2-, 4- (Fig. 13), and 8-nucleate embryo sac. Thus the mode of the embryo sac development is of the Polygonum type. An organized embryo sac has eight nuclei in seven cells: an egg cell, two synergids, two polar nuclei, and three antipodal cells (Fig. 14). The organized embryo sac is ellipsoid in shape and positioned at the upper region of the nucellus (Fig. 15). The antipodals degenerate before or soon after fertilization (Fig. 14).

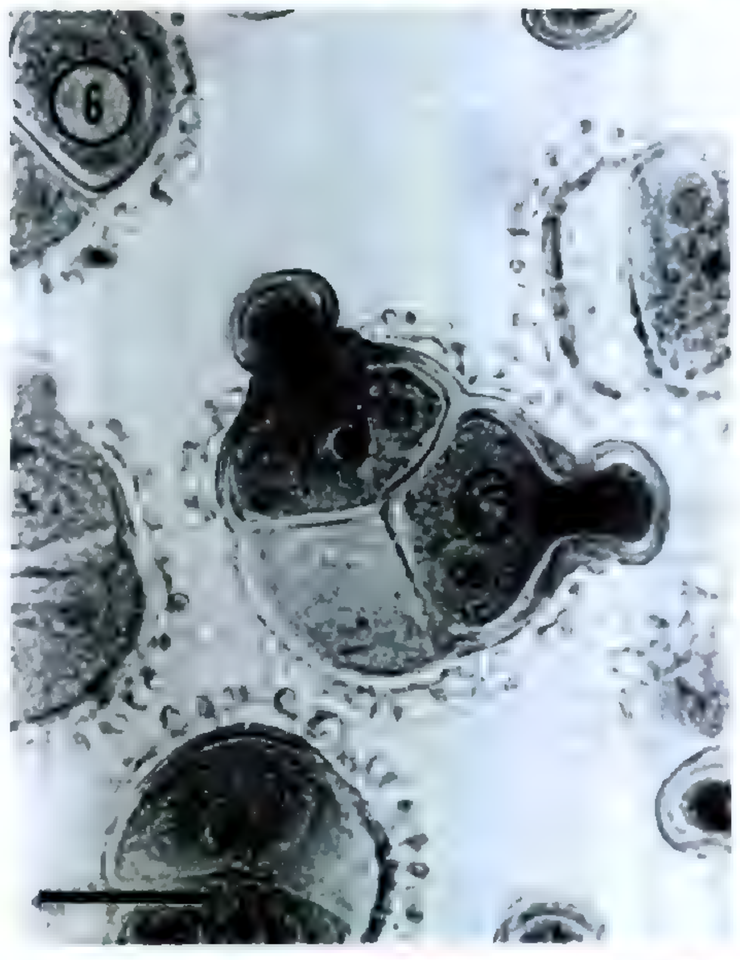
During megasporogenesis and megagametogenesis, some apical epidermal cells of the nucellus divide periclinally to form a 2-cell-layered nucellar

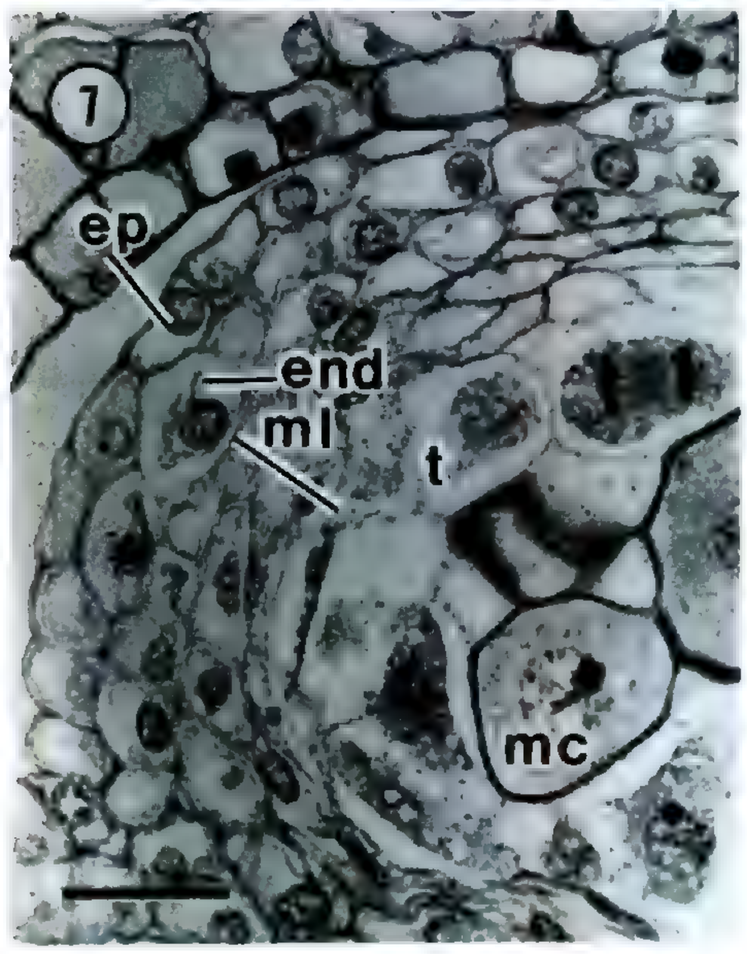


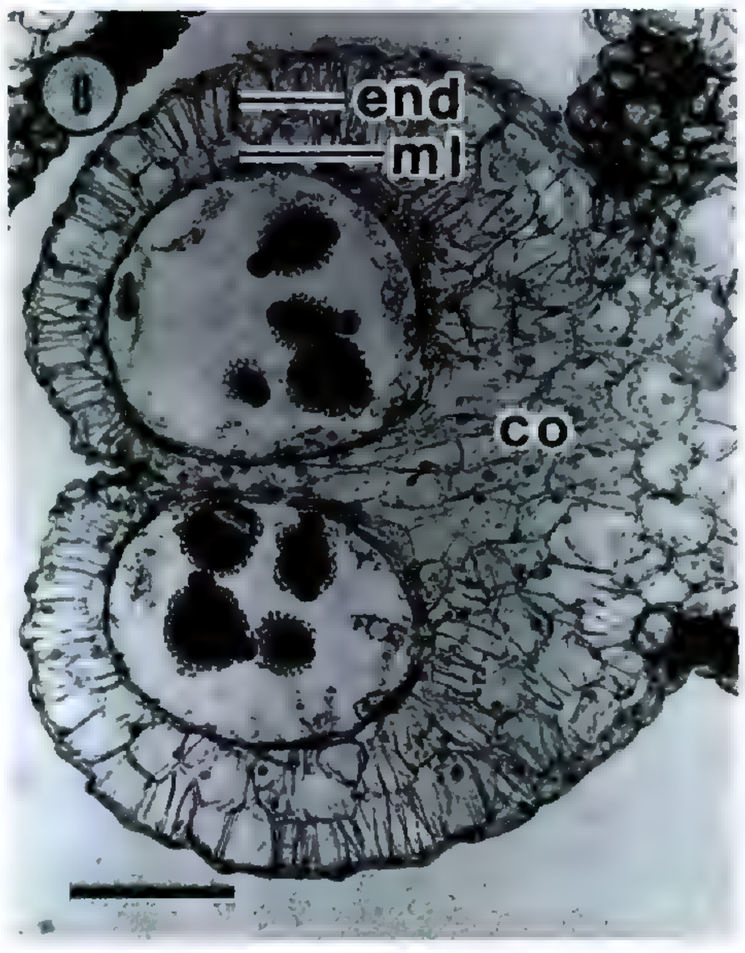




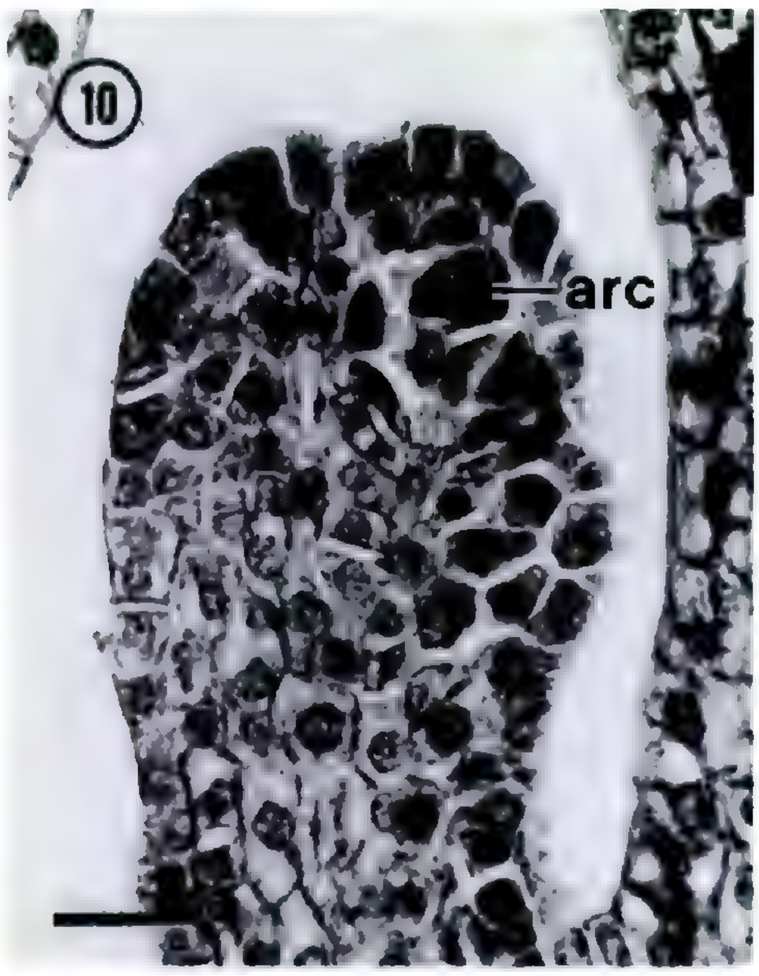


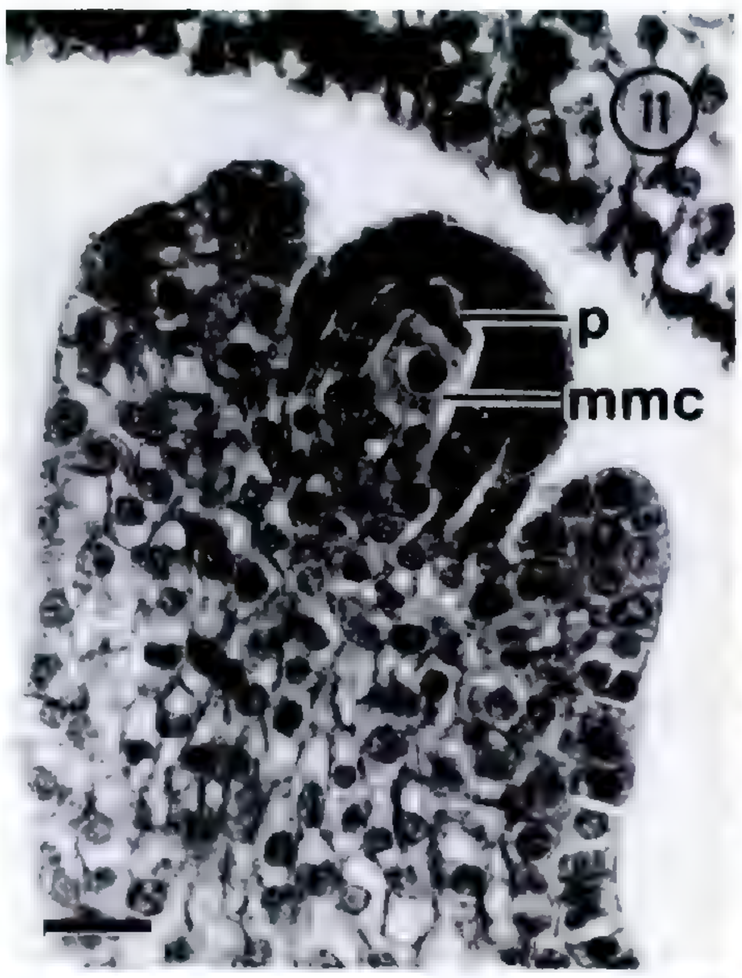


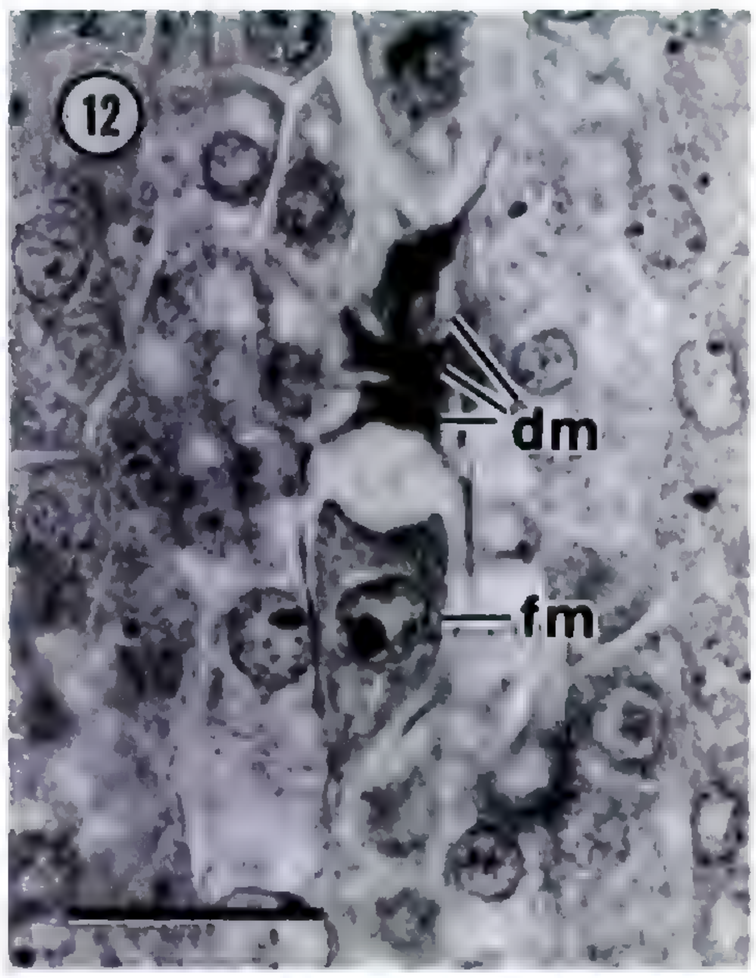


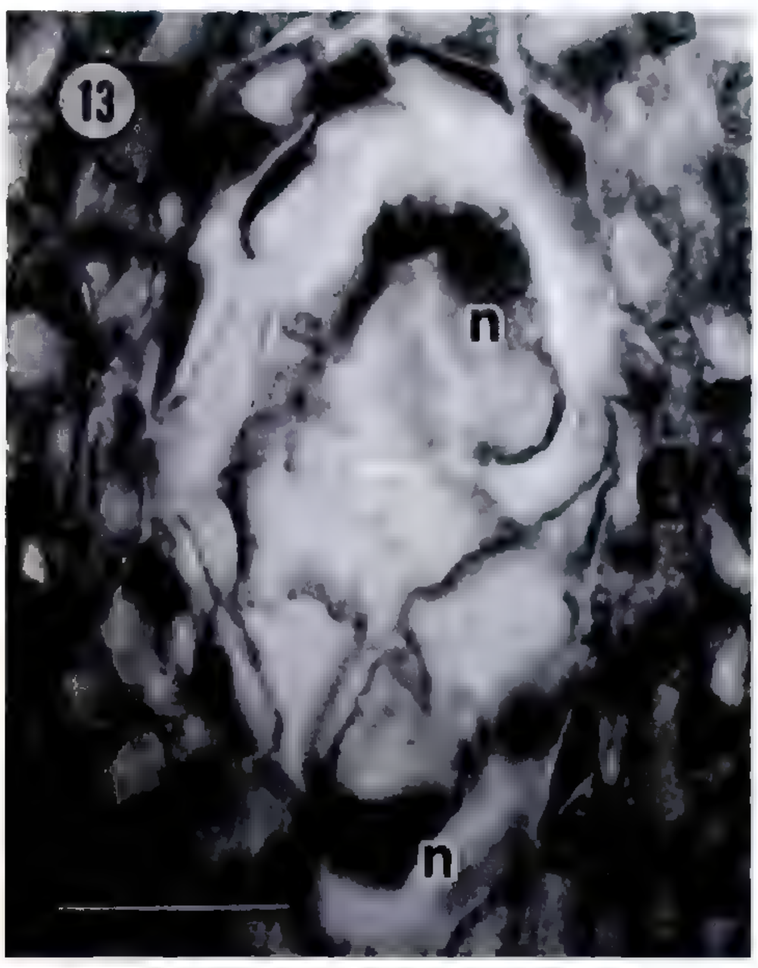


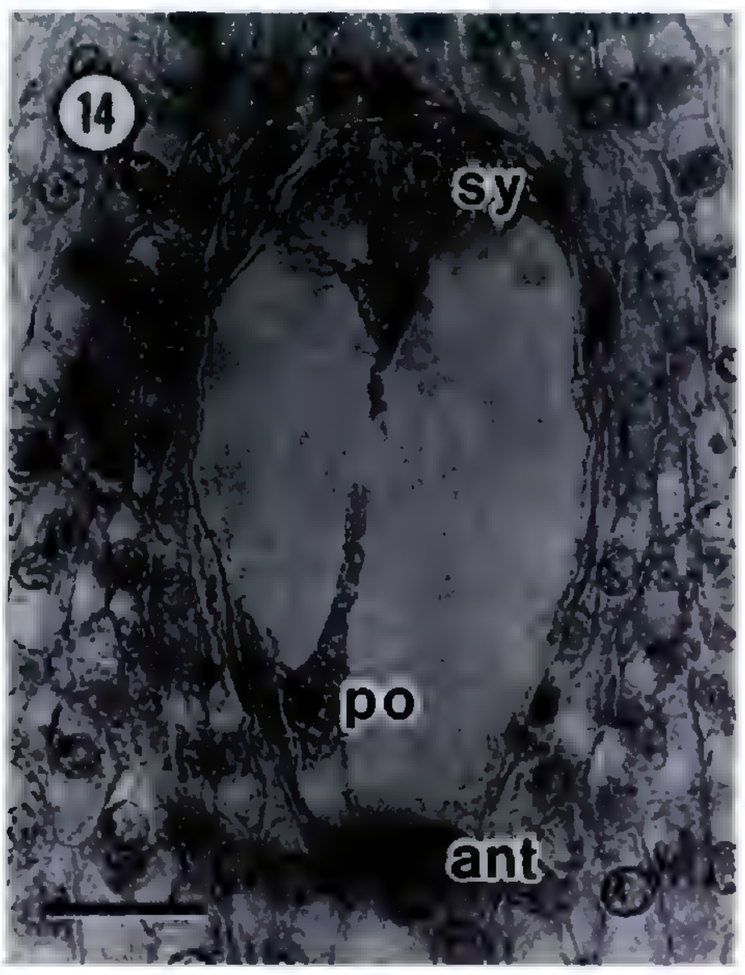
Figures 1–9. Development of anther and microspores in *Takhtajania*. —1. Longitudinal section (LS) of flower showing a small anther. —2. Transverse section (TS) of flower showing dehiscing anthers. —3. TS of anther. —4. TS of microsporangium showing 2-nucleate glandular tapetal cells. —5. Meiosis in microsporangium showing simultaneous cytokinesis. —6. Microtome sections of 2-celled mature pollen grains in tetrad. —7. Microtome section of young anther showing wall structure. —8. TS of older anther showing that fibrous thickenings develop in the outermost one of middle layers and even in part of the connective tissue as well as in the endothecium. —9. Microtome section of dehiscing anther. Abbreviations: an, anther; co, connective tissue; end, endothecium; ep, epidermis; mc, microspore mother cell; ml, middle layer; ov, ovule; t, tapetum. Scale bar equals 1 mm in Figs. 1 and 2, 100 μm in Figs. 3, 8, and 9, and 20 μm in Figs. 4–7.

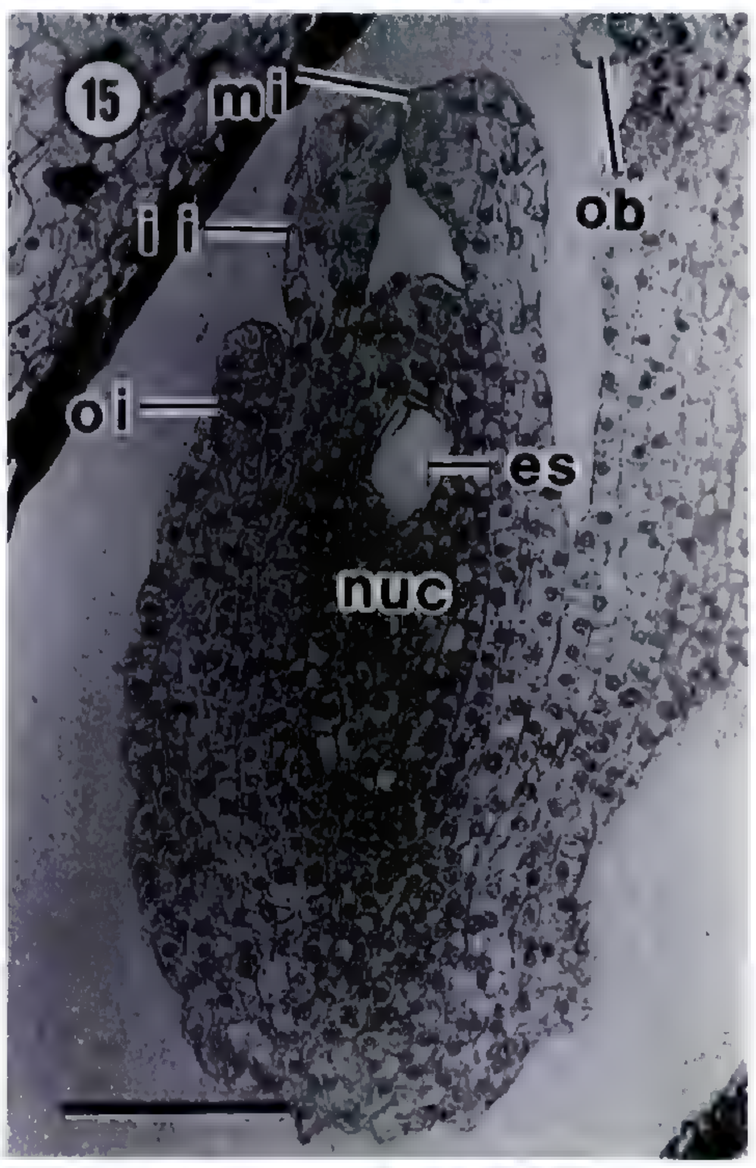


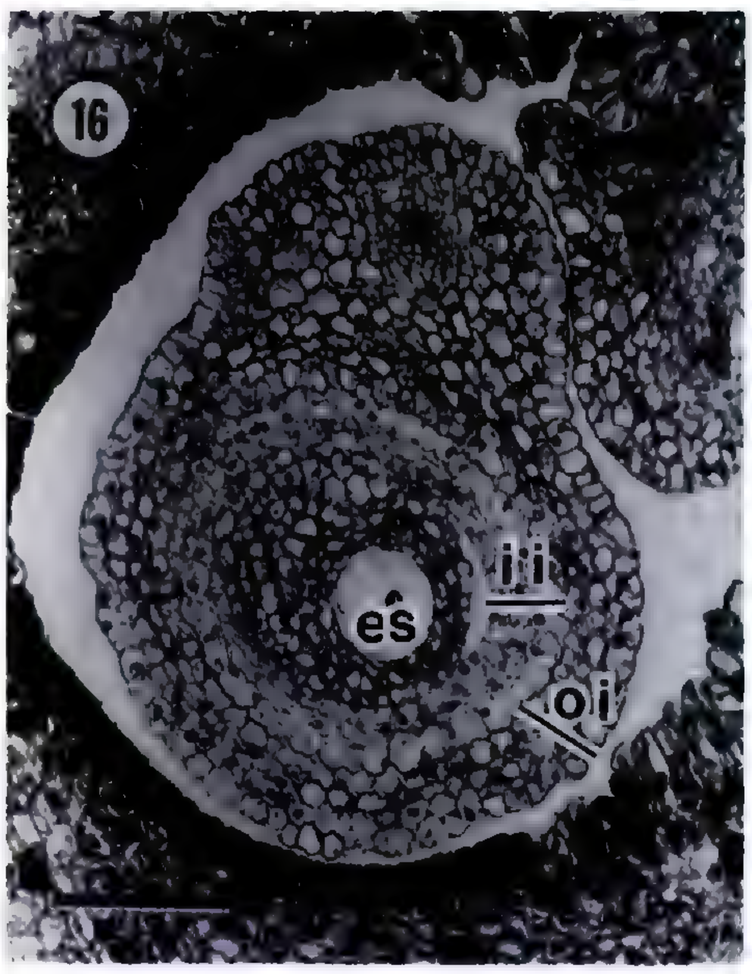


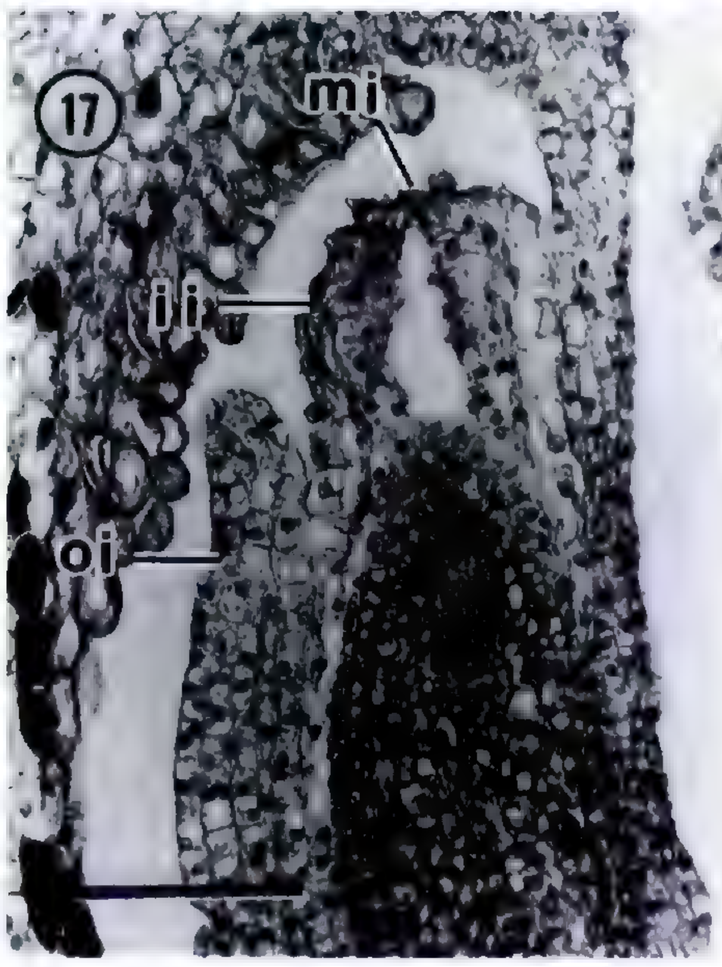


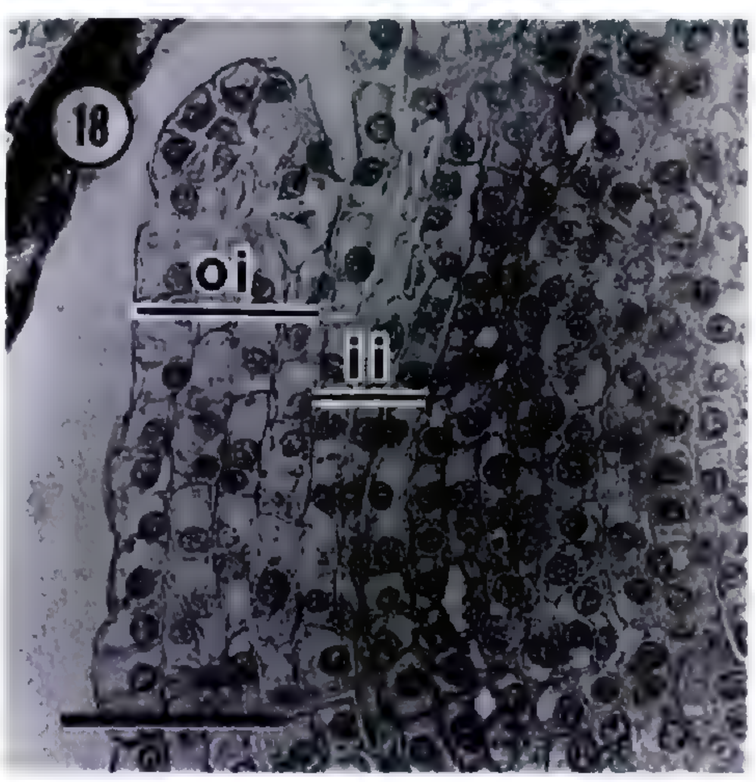




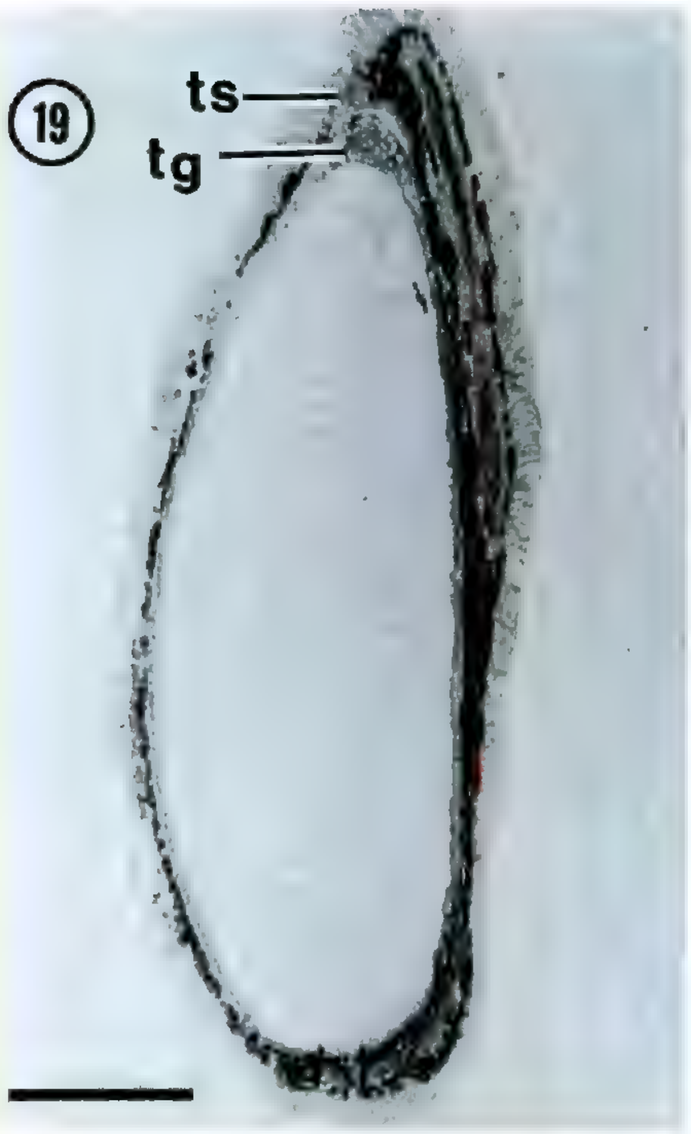


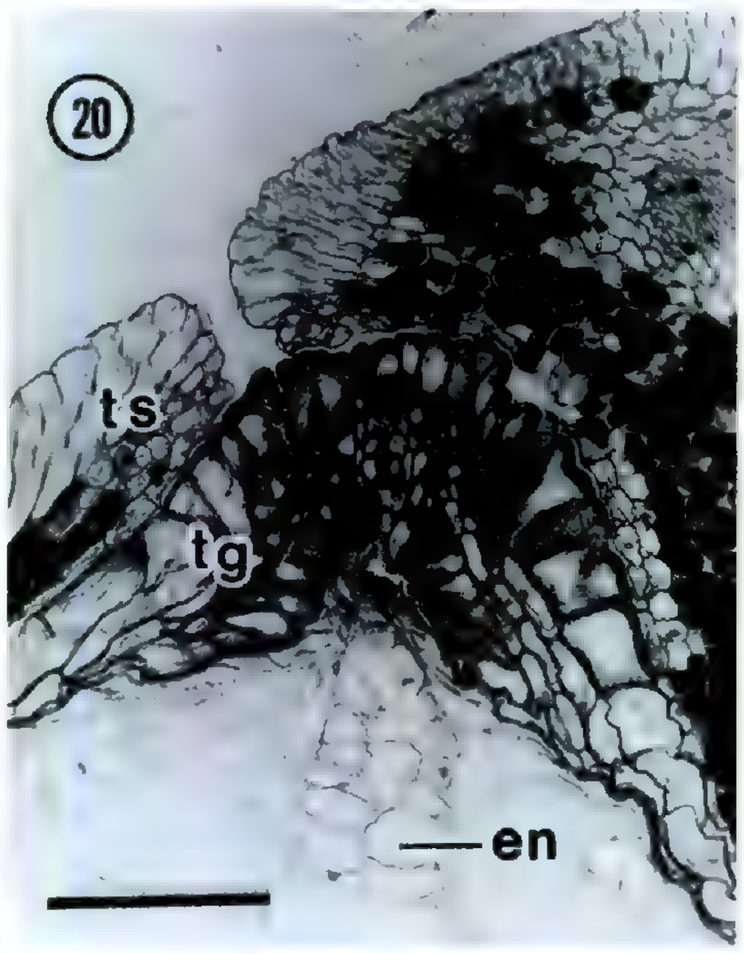


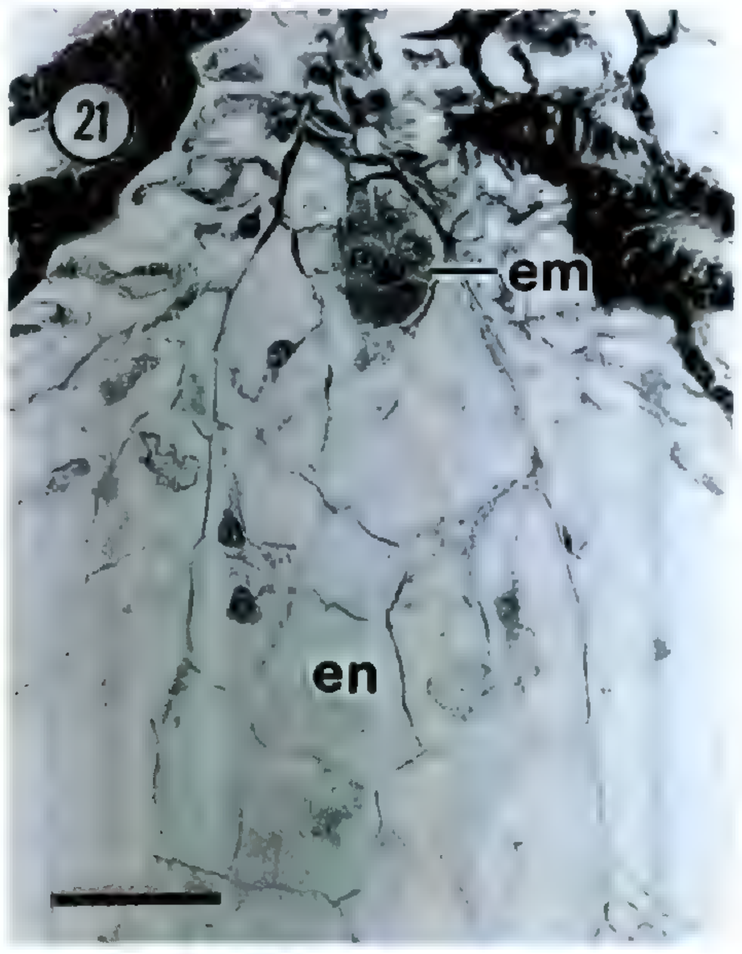


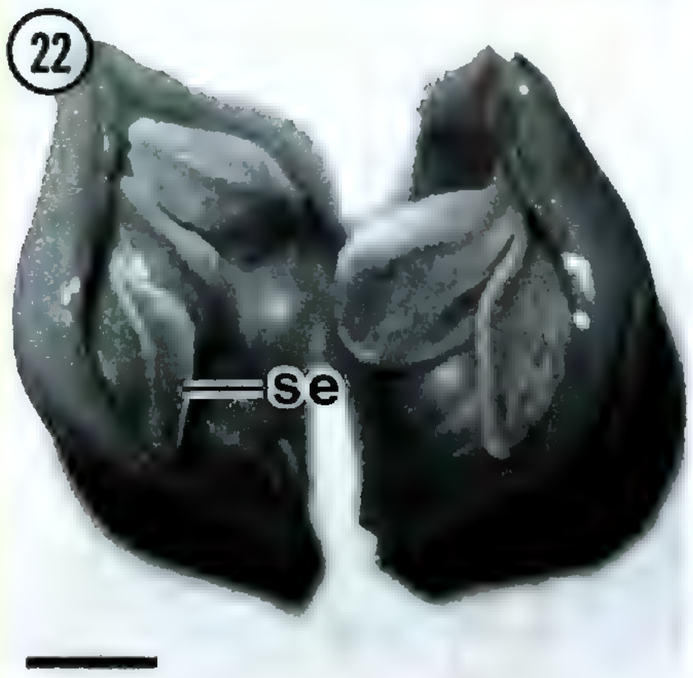


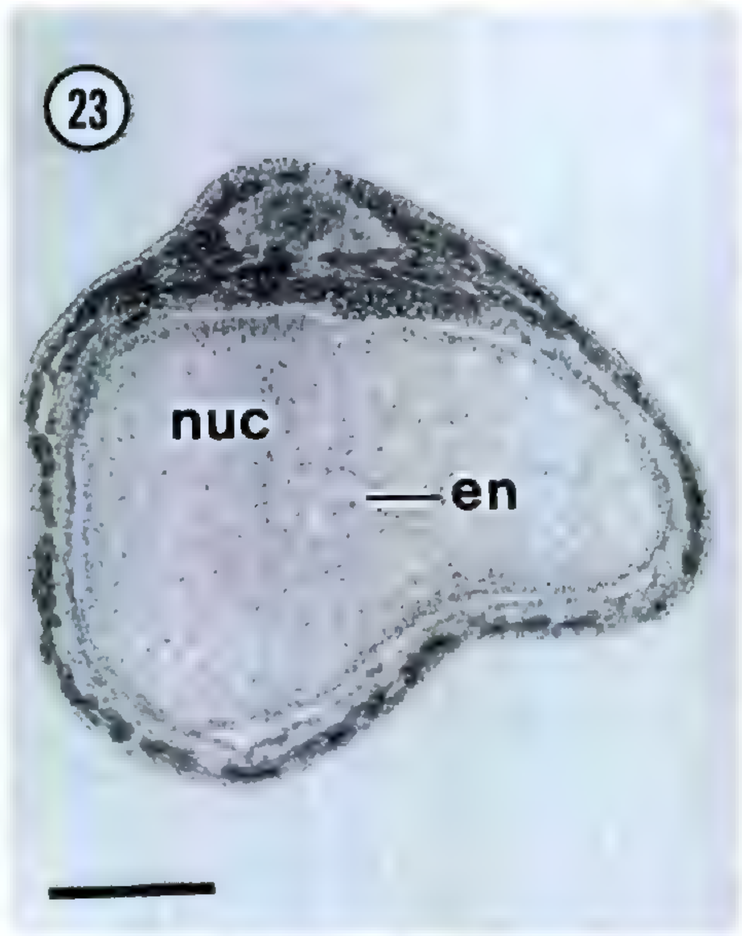
Figures 10–18. Development of nucellus, embryo sac, and integuments in *Takhtajania*. —10. Longitudinal section (LS) of ovule primordium. —11. LS of young ovule with a megaspore mother cell. —12. LS of young ovule showing a linear tetrad of megaspores, in which the chalazal megaspore is functional and the other megaspores are degenerating. —13. LS of older ovule with a 4-nucleate embryo sac, showing only two nuclei in photo. —14. LS of mature ovule with an organized embryo sac, showing one synergid, (fused) polar nuclei, and one antipodal cell in photo. —15. LS of nearly mature ovule showing the micropyle formed by the inner integument alone. —16. Transverse section of mature ovule. —17. LS of mature ovule showing the micropylar part. —18. LS of mature ovule showing the thickness of integuments. Abbreviations: ant, antipodal cell; arc, archesporial cell; dm, degenerating megaspore; es, embryo sac; fm, functional megaspore; ii, inner integument; mi, micropyle; mmc, megaspore mother cell; n, nucleus of embryo sac; nuc, nucellus; ob, obturator; oi, outer integument; p, parietal cell; po, polar nucleus; sy, synergid. Scale bar equals 500 μm in Fig. 18, 100 μm in Figs 15–17, and 20 μm in Figs. 10–14.

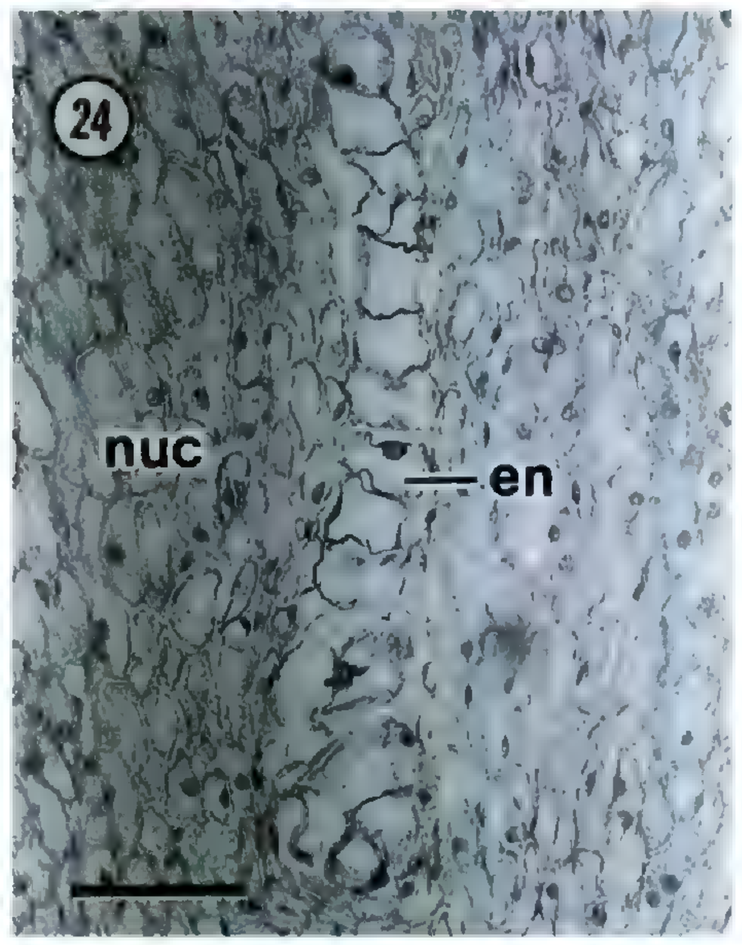


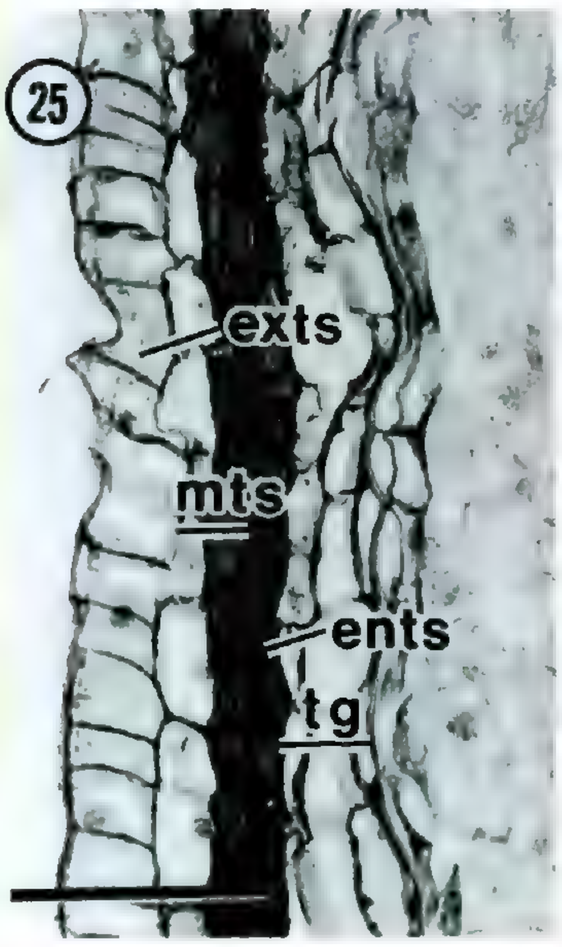


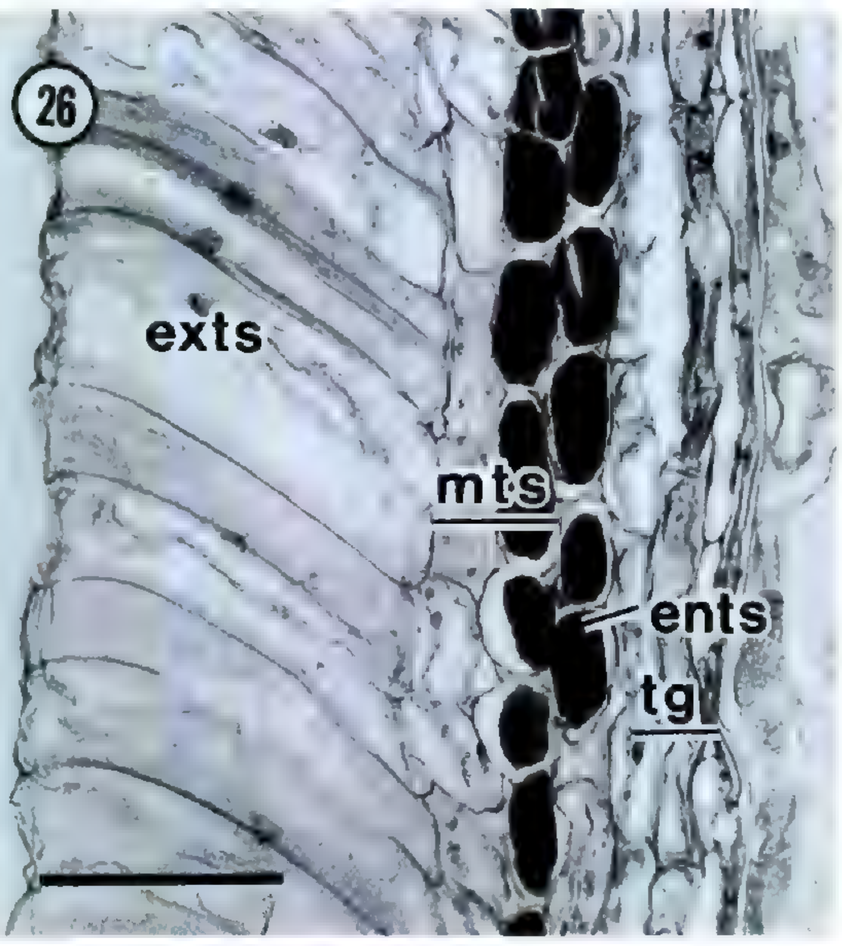


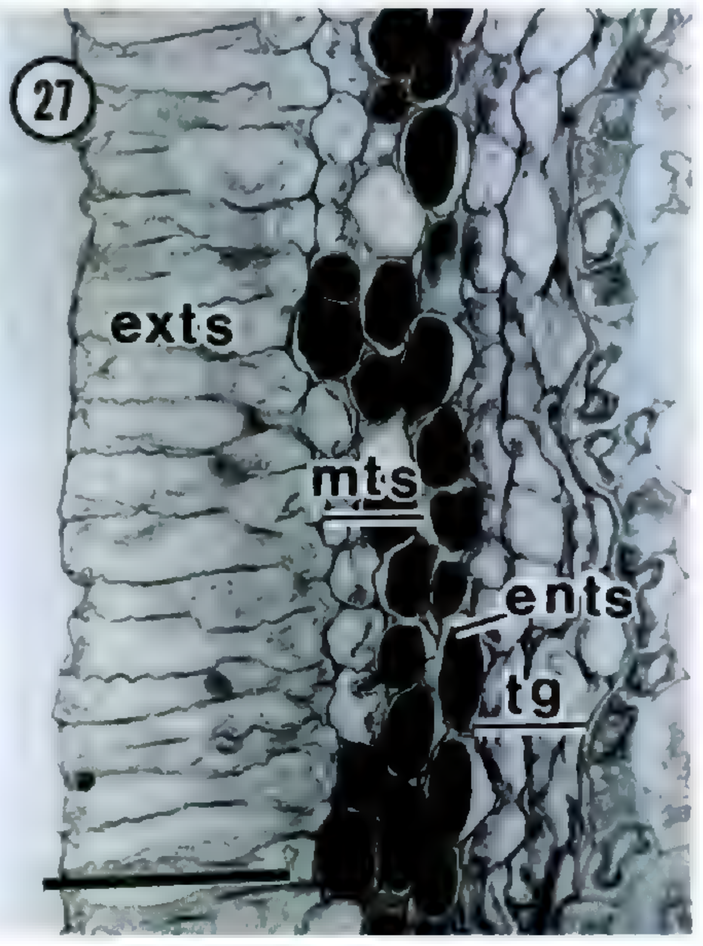












Figures 19–27. Development of seeds and seed coats in *Takhtajania*. —19. Longitudinal section (LS) of older seed. —20. Micropylar part in LS of older seed. —21. LS of older seed showing a globular proembryo and cellular endosperm. —22. Nearly mature fruit opened artificially. —23. Transverse section (TS) of young seed. —24. LS of young seed showing cellular endosperm. —25. LS of young seed coat. —26. LS of nearly mature seed coat. —27. TS of nearly mature seed coat. Abbreviations: em, proembryo; en, endosperm; ents, endotesta; exts, exotesta; mts, mesotesta; nuc, nucellus; se, seed; tg, tegmen; ts, testa. Scale bar equals 1 mm in Fig. 19, 3 mm in Fig. 22, 200 μm in Figs. 20 and 21, 100 μm in Figs. 23, 26, and 27, and 50 μm in Fig. 25.

Table 1. Comparisons of *Takhtajania* with other Winteraceae and Canellaceae in embryological characters.

Characters	Canellaceae	*Takhtajania*	Other Winteraceae[6]
Anthers and microspores			
Number of sporangia	4	4	4
Thickness of anther wall	5 cell-layered	5 or 6 cell-layered	5 or 6 cell-layered
Mode of wall formation	?	?	Basic
Anther epidermis	Persistent	Collapsed	Collapsed
Endothecium	Fibrous	Fibrous	Fibrous
Middle layers	Crushed	Crushed except fibrous outer cell-layer	Crushed except fibrous outer cell-layer
Tapetum	Glandular	Glandular	Glandular
Number of nuclei in tapetal cell	2–4, fused	2	2 (or more), fused
Cytokinesis in meiosis	Simultaneous	Simultaneous	Simultaneous
Shape of microspore tetrads	Tetrahedral	Tetrahedral	Tetrahedral
Mature pollen	2-celled	2-celled	2-celled
Ovules, nucellus, and megagametophyte			
Ovule orientation	Campylotropous[1]	Anatropous	Anatropous
Hypostase	Not formed	Not formed	Not formed
Number of archesporial cells	1	1	1
Nature of nucellus	Crassinucellate	Crassinucellate	Crassinucellate
Thickness of parietal tissue	?	5–6 cells thick	2–5 cells thick
Mode of embryo sac formation	Polygonum	Polygonum	Polygonum
Antipodal cells	Ephemeral	Ephemeral	Ephemeral
Nucellar cap	Not formed	2 cells thick	2–3 cells thick
Nucellar tissue in mature ovule	Present	Present	Present
Obturator	?	Present	Rarely present[7]
Integuments			
Number of integuments	2	2	2
Thickness of ii (early stage)	?	3(–4) cell-layered	2(–3) cell-layered
Thickness of ii (late stage)	3 cell-layered	3(–4) cell-layered	2(–3) cell-layered
Thickness of oi (early stage)	?	4–5 cell-layered	3(–4) cell-layered
Thickness of oi (late stage)	4–8 cell-layered	4–5 cell-layered	3(–4) cell-layered
Vascular bundles	?	Absent	Absent
Micropyle formation	By ii and oi	By ii	By ii[8]
Endothelium	Not formed	Not formed	Not formed
Endosperm and embryo			
Mode of endosperm formation	?	*ab initio* Cellular	*ab initio* Cellular
Type of embyrogeny	?	?	Unknown, irregular type (*Drimys winteri*)
Seed and seed coat			
Cells of endostome after fertilization	Unspecialized	Persistent, thick-walled; outermost cells enlarged	Persistent, thick-walled; outermost cells enlarged
Pachychalazy	No (except *Cinnamosma*)[2]	No	No
Aril/wing	Not formed[3]	Not formed	Not formed
Endosperm in mature seed	Copious, ruminate[4]	Copious	Copious
Type of seed coat	Exotestal[5]	Exotestal	Exotestal
Thickness of testa	?	4–5 cell-layers thick	4–6 cell-layers thick
Cells of exotesta	Lignified[5]	Lignified, palisadal	Lignified, palisadal
Cells of mesotesta	Unspecialized or oily idioblasts	Rectangular, tanniniferous	Little specialized
Cells of endotesta	Unspecialized	Rectangular, tanniniferous	Unspecialized, crushed
Cells of tegmen	Crushed	Unspecialized	Crushed, or rarely tangentially elongate, thick-walled

Table 1. Continued.

Characters	Canellaceae	*Takhtajania*	Other Winteraceae[6]
References	Corner (1976), Parmeswaran (1961, 1962)	Present study	Bhandari (1963), Bhandari & Venkataraman (1968), Corner (1976), De Boer & Bouman (1974), Prakash et al. (1992), Sampson (1963), Smissen (1993), Swamy (1952), Vink (1970, 1977)

Abbreviations: ii, inner integument; oi, outer integument.

[1] Ovules of Canellaceae are described as anatropous (Netolitzky, 1926; later authors). However, a drawing of a longitudinal section of an ovule of *Warburgia stuhlmannii* Engl. (Parameswaran, 1962, fig. 25 on p. 173) suggests that the ovule is campylotropous rather than anatropous.

[2] According to Corner (1976: 86), the description of Parameswaran (1961) suggests that the large seed of *Cinnamosma* appears to be pachychalazal, but this must be confirmed.

[3] According to Corner (1976: 86), "Baillon described a vestigal aril round the hilum of *Canella*, but later authors have not reported it."

[4] According to Parameswaran (1961), *Cinnamosma* (*C. macrocarpa* and *C. madagascariensis*) has ruminate processes in seed coats, which are not known in *Canella, Cinnamodendron* (including *Capsicodendron*), and *Warburgia*.

[5] Based on earlier observations, Corner (1976: 86) noted that the exotesta is composed of sclerosed cells (except *Cinnamosma*), and that the seeds appear exotestal. The structure of the seed coat of Canellaceae has been studied very poorly, and more detailed study of the seed coat is needed for critical comparison.

[6] The following taxa have been studied for some or part of the embryological characters of the genera of Winteraceae other than *Takhtajania*: "*Belliolum haplopus*" (B. L. Burtt) A. C. Smith (Corner 1976), *Drimys* (*Tasmannia*) *piperita* Hook. f. (Corner, 1976), *D. winteri* (Bhandari & Venkataraman, 1968; De Boer & Bouman, 1974), *Pseudowintera colorata* (Raoul) Dandy (Bhandari, 1963; Corner, 1976), *P. axillaris* (J. R. Forst. & G. Forst.) Dandy (Sampson, 1963), three species of *Tasmannia* (Prakash et al., 1992), *Zygozynum bailloni* Tiegh. (Swamy, 1952), and *Z. stipitatum* Baill. (syn. *Exospermum stipitatum*) (Smissen, 1993). Unless otherwise stated, embryological features presented above are common to all the taxa studied.

[7] The presence of an obturator is reported in all three species of *Tasmannia* studied by Prakash et al. (1992) but is not clear in other genera.

[8] According to Corner (1976: 282) in "*Belliolum haplopus*" the micropyle is formed by the endostome and the exostome. This is reiterated by Johri et al. (1992: 242), who state that "in *Zygozynum* both the integuments are concerned" in the formation of the micropyle. However, there is no clear evidence for this, and all other literature sources available for this character in Winterceae indicate that the micropyle is formed by the inner integument alone and is covered by the outer integument (testa) after fertilization.

cap. Cells of the nucellus remain around the mature embryo sac (Fig. 15). An obuturator is formed near the micropyle (Fig. 15). A hypostase is not formed.

INTEGUMENTS

The ovule is bitegmic, having an inner and an outer integument (Figs. 15–18). Neither the inner nor outer integument are multiplicative. The inner integument is 3-cell-layered (Fig. 18) from the beginning to older stages of development except at the apical part where it is about 5- to 7-cell-layers thick (Fig. 15). Some cells of the inner epidermis of the inner integument may, however, divide in places to increase the thickness, so that the inner integument is 4-cell-layered in places. The outer integument is 4- to 5-cell-layered from the beginning to older stages of ovules (Fig. 18).

By the fertilization stage the inner integument always develops beyond the outer integument to cover the tip of the nucellus, so that the micropyle is formed by the inner integument alone (Figs. 15, 17). However, after fertilization, the testa (developed outer integument) develops further and reaches the tip of the tegmen (developed inner integument) (Figs. 19, 20). Therefore, when looked at in seeds, the micropyle appears to have been formed by both the inner and the outer integument.

ENDOSPERM AND EMBRYO

Fertilization is porogamous. Endosperm formation *ab initio* is of the Cellular type. No free endosperm nuclei have been observed in young seeds. Compared to the whole size of the nucellus, which is enlarged after fertilization, the embryo sac composed of cellular endosperm is small and narrow (Figs. 20, 21). In nearly mature or the oldest seeds available, the endosperm is positioned in the center

of the seed and is surrounded by a massive nucellar tissue, which appears to be degenerating (Fig. 19).

We did not examine embryogenesis in detail in this study, but fragmentary observations of early and late embryogenesis indicate that it proceeds normally to form a globular embryo (Fig. 21). In the nearly mature or oldest seeds available, we could not observe a dicotyledonous embryo. The seeds seem to be released from a fruit before the globular embryo develops into a dicotyledonous stage.

SEED AND SEED COAT

Nearly mature seeds are ellipsoid and somewhat angular (Figs. 19, 22) without any appendages such as an aril or a wing. The seed coat is formed by both the tegmen and the testa. Both the tegmen and the testa do not increase their thickness after fertilization. The tegmen is 3- to 4-cell-layers thick, and the testa is 4- to 5-cell-layers thick (Fig. 25). As the seed develops, cells of the exotesta radially elongate to assume a palisadal structure, while 3 to 4 underlying cell-layers of the mesotesta and the endotesta are rectangular in shape and tanniniferous (Figs. 25–27). On the other hand, none of the cell-layers of the tegmen exhibit any particular specialization and probably degenerate or collapse except at the micropylar part. Cells of the micropylar part of the tegmen are variously enlarged and thick-walled to form a persistent cap-like structure (Fig. 20). The seed coat is thus exotestal (for terminology of seed coat types, see Corner, 1976; Schmid, 1986).

Discussion

In Table 1 embryological features of *Takhtajania* are summarized in comparison with those of the other Winteraceae as well as of the Canellaceae. Embryologically *Takhtajania* agrees well with the four other genera of Winteraceae (*Drimys*, *Pseudowintera*, *Tasmannia*, and *Zygogynum* s.l.). No particular characters suggest the distinctness of *Takhtajania* from the other Winteraceae and its basal position within the family. The embryological features of the family are summarized as follows.

Anther tetrasporangiate; anther wall development of the Basic type (unknown in *Takhtajania*); the anther epidermis becomes collapsed; the endothecium, the outermost cell-layer of the middle layers, and occasionally part of the connective tssue developing fibrous thickenings; other middle layers degenerating, and tapetum glandular; tapetal cells 2-nucleate; cytokinesis in meiosis simultaneous; the predominant shape of microspore tetrads tetrahedral; mature pollen grains 2-celled.

Ovule anatropous, bitegmic, and crassinucellate; archesporium 1-celled; parietal tissue 5–6 cells thick, tetrads of megaspores linear or T-shaped; mode of embryo sac formation of the Polygonum type; antipodal cells ephemeral; the mature embryo ellipsoid and positioned at the upper region of an enlarged nucellus; 2-cell-layered nucellar cap formed; hypostase not differentiated; an obturator formed; ovule or seed not pachychalazal; inner integument 3(–4) cells thick, and outer integument 4–5 cells thick; both integuments not multiplicative; vasculature absent in both the integuments; endothelium not formed; micropyle formed by the inner integument alone, but covered by an exostome after fertilization.

Fertilization porogamous; endosperm formation of *ab initio* Cellular type; mode of embryogeny of an undetermined type (in *Drimys winteri* J. R. Forst. & G. Forst.). Mature seeds albuminous with a very small embryo; seed coat exotestal, having lignified palisadal exotestal cells to form the most specialized mechanical structure; cells of mesotesta and endotesta little specialized or crushed but rectangular and tanniniferous in *Takhtajania*; tegmen usually crushed.

COMPARISIONS WITH CANELLACEAE

Based on embryological data that is available for Canellaceae, the Winteraceae resemble Canellaceae in general, including having an exotestal seed coat (Table 1). Based on the presence of lignified exotestal cells (except in *Cinnamosma*), Corner (1976: 86) stated that the seeds of Canellaceae appear exotestal and indicate alliance with the Winteraceae. In addition, according to Huber (pers. comm. in Kubitzki, 1993), seeds of *Canella alba* Nees are exotestal, with a collapsed tegmen and nucellus, abundant and thin-walled endosperm free of starch, and a small and slender embryo, and they agree with those of Winteraceae (and Illiciales). However, information on the seed coat structure of Canellaceae is still extremely limited for critical comparison. Although the seed coat structure of several species of *Canella* and *Cinnamosma* has been briefly summarized (Corner, 1976), its development has never been documented. Therefore the exact structure of any species of the Canellaceae is not clear enough to verify how the seed coat structure of the Winteraceae resembles or is different from that of Canellaceae. Nor can we clarify whether or not the two families share exactly distinct seed coat structure from that of related taxa such as Piperales sensu lato (Qiu et al., 1999). Canellaceae need further detailed studies of these features.

Despite the limited data available for the Canellaceae, the whole family Winteraceae is clearly distinct from it, and all the five genera of the family share a few characteristic features that are not found in Canellaceae. For instance, in Winteraceae fibrous thickenings occur in the outermost one of the middle layers (and occasionally even in part of the connective tissue) as well as in the endothecium, while they occur only in the endothecium in Canellaceae; the micropyle is formed by the inner integument alone in Winteraceae, but is formed by both the inner and outer integuments in Canellaceae; the exostome is formed after fertilization in Winteraceae and not by the fertilization stage as in Canellaceae; ovules are anatropous in Winteraceae but campylotropous in Canellaceae; seeds of Winteraceae have a persistent micropylar part of the tegmen composed of variously enlarged, thick-walled cells, which is not reported in Canellaceae; the exotesta is palisadal and formed by radially enlarged, lignified exotestal cells in Winteraceae but not palisadal in Canellaceae. These distinctive embryological features support the coherence of the Winteraceae including *Takhtajania* and highlight the distinctness of the Winteraceae from the Canellaceae.

As discussed above, there is no embryological evidence to distinguish *Takhtajania* from the remainder of the family. In other words, Winteraceae are a well-defined group embryologically. Ancestral Winteraceae date back to the Barremian stage of the Early Cretaceous in tropical Northern Gondwana, and their descendents migrated from there to the southern temperate zone and eventually to the current distribution areas (Doyle, 2000 this issue). However, despite its modern widespread distribution, the family has been little diversified in embryological characters since it diverged from a common ancestor with Canellaceae.

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NOTES ON THE VASCULAR ANATOMY OF THE FRUIT OF *TAKHTAJANIA* (WINTERACEAE) AND ITS INTERPRETATION[1]

Thierry Deroin[2]

ABSTRACT

During fruit set the vascular skeleton of the *Takhtajania* gynoecium did not alter fundamentally, but there is a transfer of role in ovule nutrition from the median carpel bundles to meridian-lateral and partly lateral ones, in accordance with a pattern previously recognized in Annonaceae and Magnoliaceae. Lack of sclerification (only some phloem fibers) of secondary tissues and weak lengthening of stipe demonstrate the paedomorphic character of this genus among the Winteraceae. This trait is correlated with wider morphogenetic potentialities: syncarpy and likely dehiscence.

Key words: ovary, paedomorphy, paracarpy, *Takhtajania*, winteroids.

About 20 years ago, the description of bicarpellate syncarpy in *Takhtajania perrieri* (Capuron) Baranova & J.-F. Leroy from Madagascar (Leroy, 1977, 1978, 1980; Vink, 1978) catalyzed a phase of research and analysis regarding the evolution of the gynoecium of Winteraceae and of Magnoliales in general. More recently, Leroy (1993) presented a brief synthesis of the debate resulting from this discovery, which was all the more interesting since the information available at the time gave him every reason to believe that the genus *Takhtajania* was extinct. However, after considerable effort, Malagasy foresters and their colleagues from the Missouri Botanical Garden found living plants of *T. perrieri* in 1997, and thereby made it possible to undertake a study of the vascular anatomy of the fruits of this species. The results presented here thus represent an unexpected continuation of an earlier study of the gynoecium of this plant (Deroin & Leroy, 1993).

MATERIALS AND METHODS

The fruits examined in this study were collected by C. Birkinshaw (*483*, MO) in the Anjanaharibe-Sud reserve. They were fixed in the field in FAA and then transferred in the lab to a glycerol:ethanol:water solution for storage. One fruit was dehydrated using tertiary butanol and embedded in paraffin (melting point = 60°C) using standard procedures (Gerlach, 1984), and then cut into 12 μm transverse sections, which were stained successively with 0.5% aqueous Astrablue and 10% Ziehl's fuchsin, and then dehydrated using acetone and mounted in Eukitt.

A similar protocol was used for an older flower from the type material (*Perrier de la Bâthie 10158*, P) after restoration using 10% aqueous ammonia, postfixation in FAA, and the preparation of 8 μm sections (Deroin & Leroy, 1993). Comparison of the material from these two sources was thus facilitated. Photographs were prepared using a Zeiss photomicroscope with an orange filter and Ilford Pan F Plus 50 ASA film.

OBSERVATIONS

The material available contained only the later stages of fruit development (Fig. 1). The series of fruits showed that: (1) no growth, either in length or thickness, occurs outside the gynoecium; (2) a basal stipe(s) observed in the fruit must have formed during an earlier stage, as little if any further elongation can be seen in the late series studied here, and the stipe is not yet differentiated at anthesis (Vink, 1978), although its vascularization can already be detected (Deroin & Leroy, 1993); (3) the ovary locule (l) does, however, grow considerably, and is asymmetrical in the plane of the two carpels, one of which elongates more than the other.

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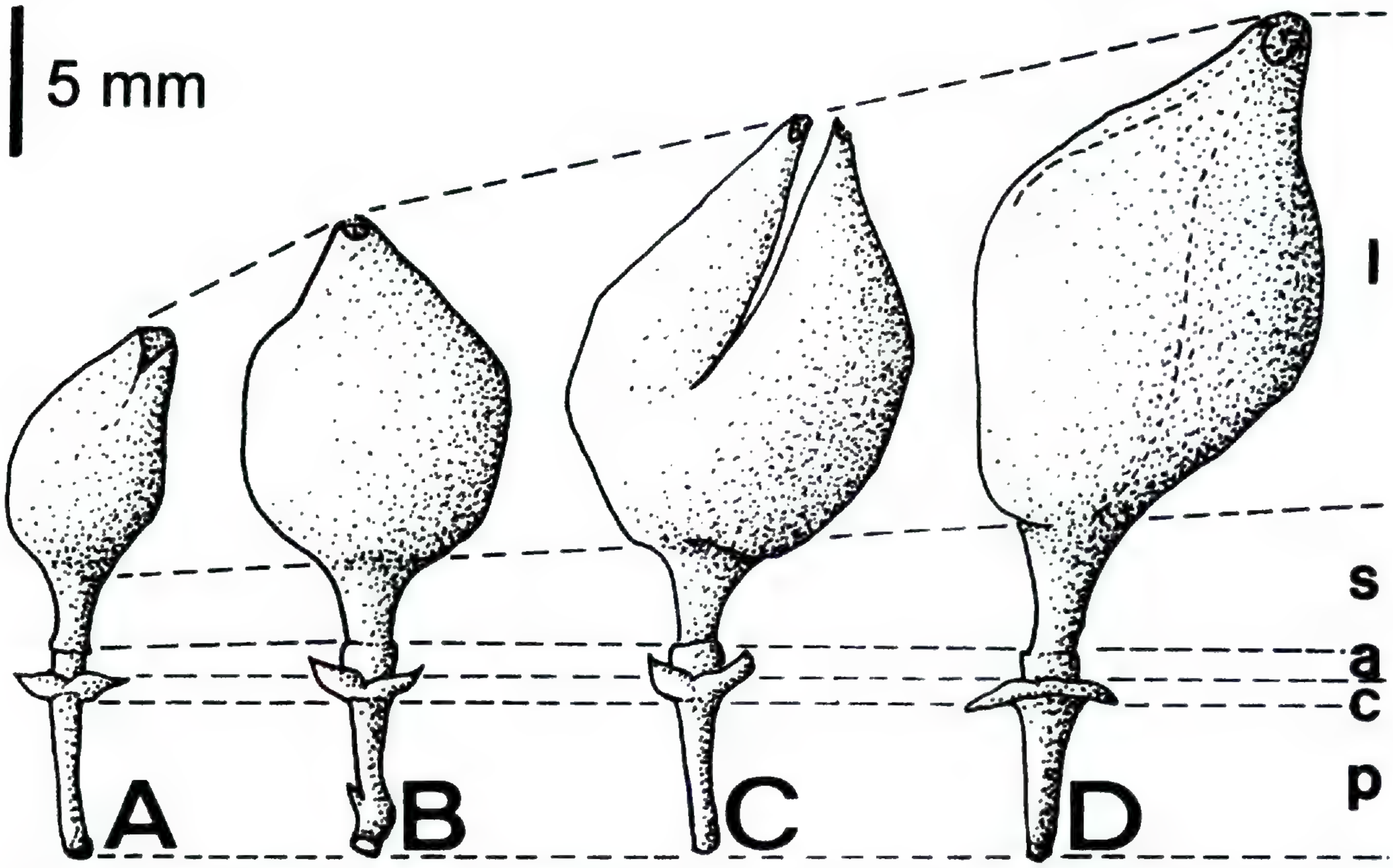


Figure 1. Four developmental stages of *Takhtajania* fruit. In the sample studied, dehiscence (C) can take place before maximum size is reached. a: androecial zone; c: calyx and corolla zone; l: area of the ovary locules; p: pedicel; s: stipe.

This differential growth could explain the rather irregular dehiscence that proceeds downward from the stigmatic scars.

The anatomy of the pedicel and receptacle of the fruit does not differ substantially from that of the flower (Fig. 2). The highly elliptic stele, with 15 vascular strands (Fig. 2A), gives rise to 2 median sepals (Fig. 2B, C), and then their lateral bundles (Fig. 2D–G). The stele, which then becomes circular and broadly medullate in cross section (Fig. 2H), gives rise farther up (Fig. 2I, J) to all of the separate traces feeding the petals and stamens (ca. 27 in all). The histological changes that take place following anthesis are minimal: lignification of the xylem (vessels stainable with fuchsin) and formation of subero-phellodermal strata at the level of the corolla and the androecium. No secondary phloem-xylem structures form.

At the extreme base of the stipe (Fig. 2K) the gynoecium appears to lack cork, and the stele divides (Fig. 2L–O) in connection with the dorsal sutures of the carpels. Higher in the stipe (Fig. 2P–S) the stele condenses in the plane of the sutures, forming two symmetrical lines of bundles. Two divided median carpellary bundles can thus be recognized, as often seen in Winteraceae, which are fused to the meridian-lateral bundles to form median complexes (mc) and the two synlateral bundles (sl), which are relatively narrower at this level. The stele then becomes more complex (Fig. 2T) by giving rise to meridian-lateral traces, whereas the sutures extend far to the interior by a well-differentiated epidermis (Fig. 4C). The center is obliterated by large-celled parenchyma, although at anthesis this level is hollow (Deroin & Leroy, 1993, figs. 1, 3).

At the base of the locule in the fruit (Fig. 2U) the lateral and median-lateral vascularizations are much more developed than the median carpellary complexes, whereas the inverse is true at anthesis. Up to the mid-level of the locule, these divided median complexes give rise on each side to 3 median-lateral bundles (1, 2, and 3, Fig. 3A–C), whereas the lateral carpellary bundles are generally separate (Fig. 3A–E, l1, and l2).

Fruit dehiscence proceeds from the top down as the wall is torn either between the separated laterals or next to the synlateral. No specialized tissues can be seen at this level (Fig. 4F). The vascularization at the top of the fruit is formally identical to that observed in the ovary (Fig. 5A, C, E): meridian-lateral traces 1 and 2 fuse back with the laterals to form the placental bundles (pl, Fig. 5B, D). It should be noted, however, that pl2 is comprised only of the median-lateral traces 1 and 2 of carpel 2. The median-lateral traces 3 terminate

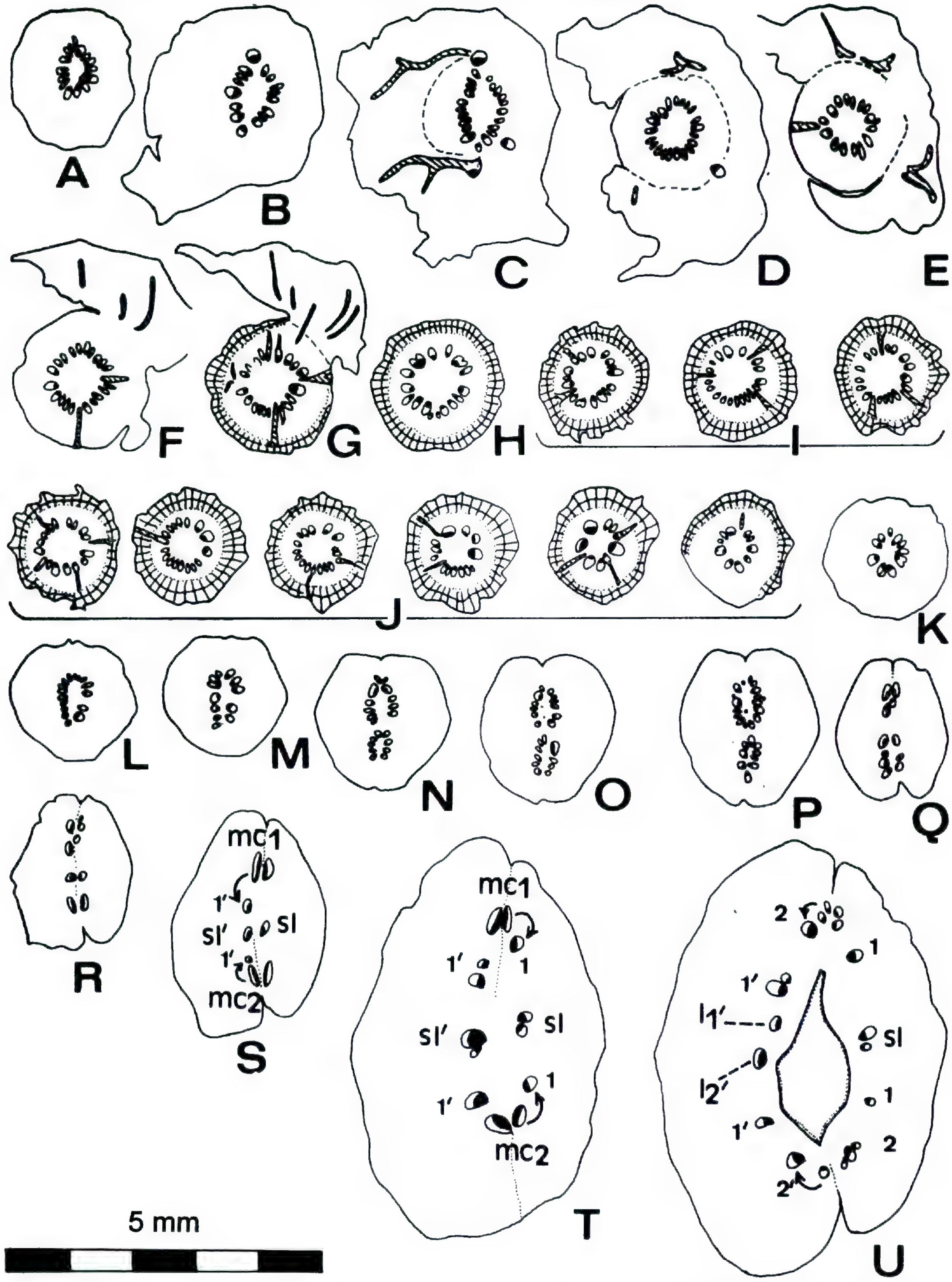


Figure 2. Ascending sections of *Takhtajania* fruit. —A. Pedicel. —B–G. Perianth. —H–J. Androecium (note the peripheral cork). —K–T. Stipe. —U. Extreme base of the ovary locule. l: lateral bundle; mc: median complex; sl: synlateral; 1, 1′, 2, 2′, median-laterals.

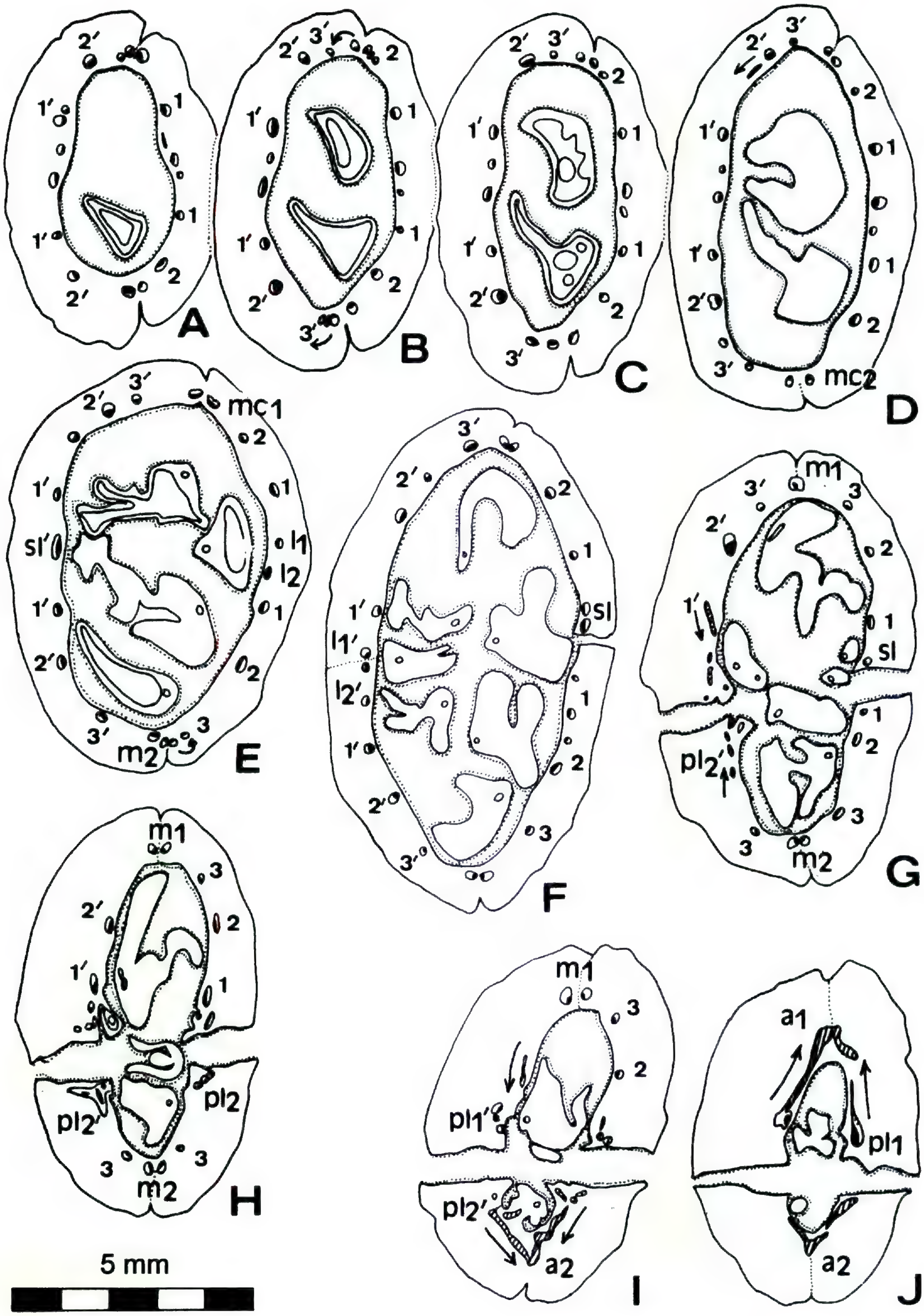
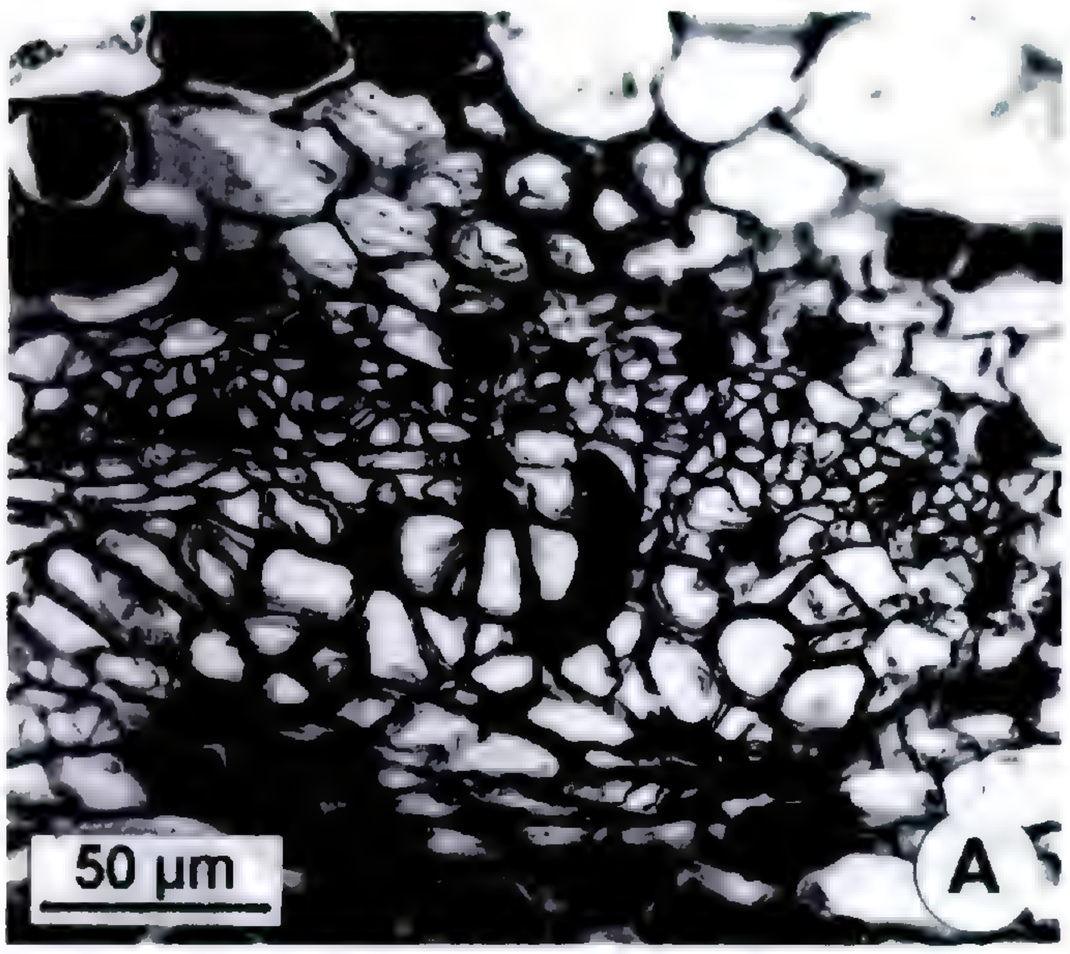
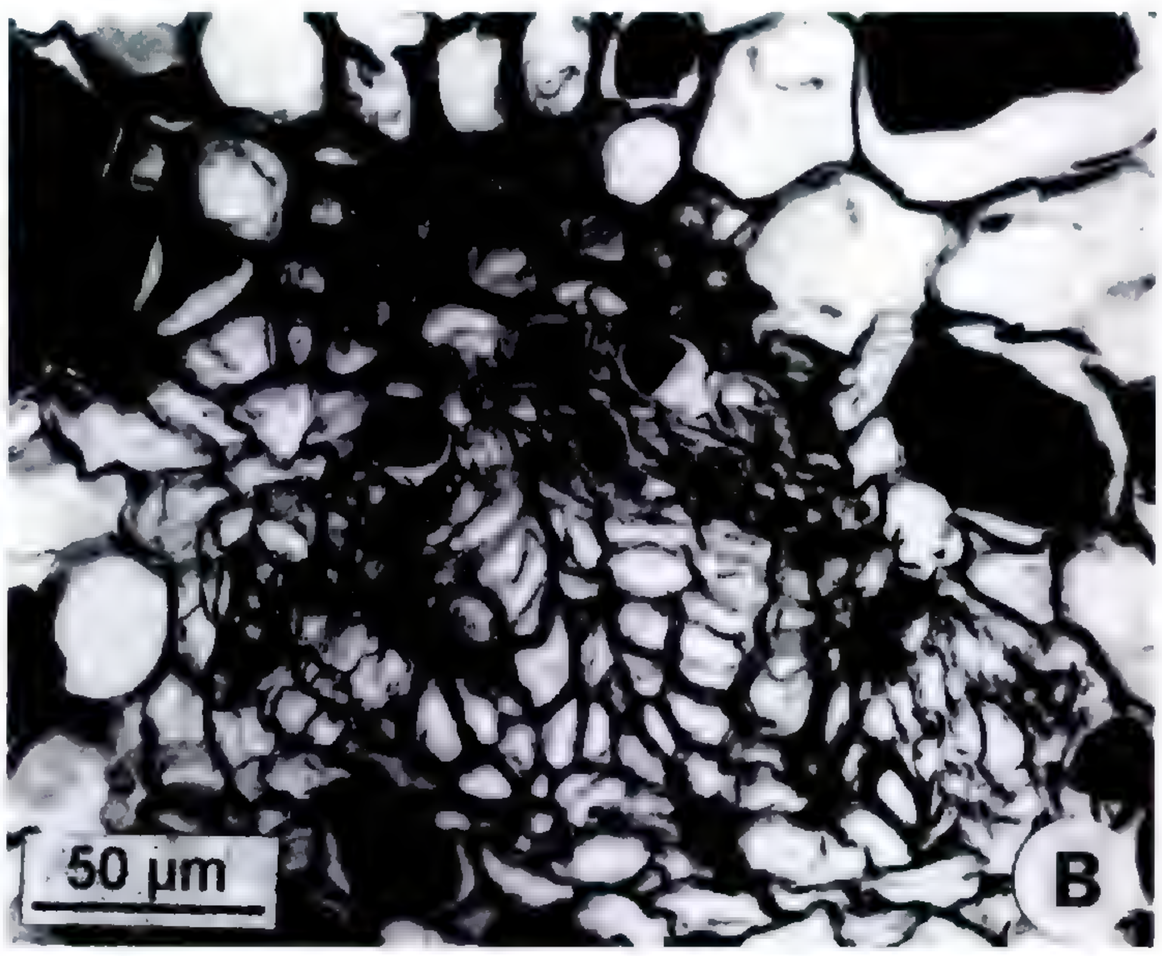
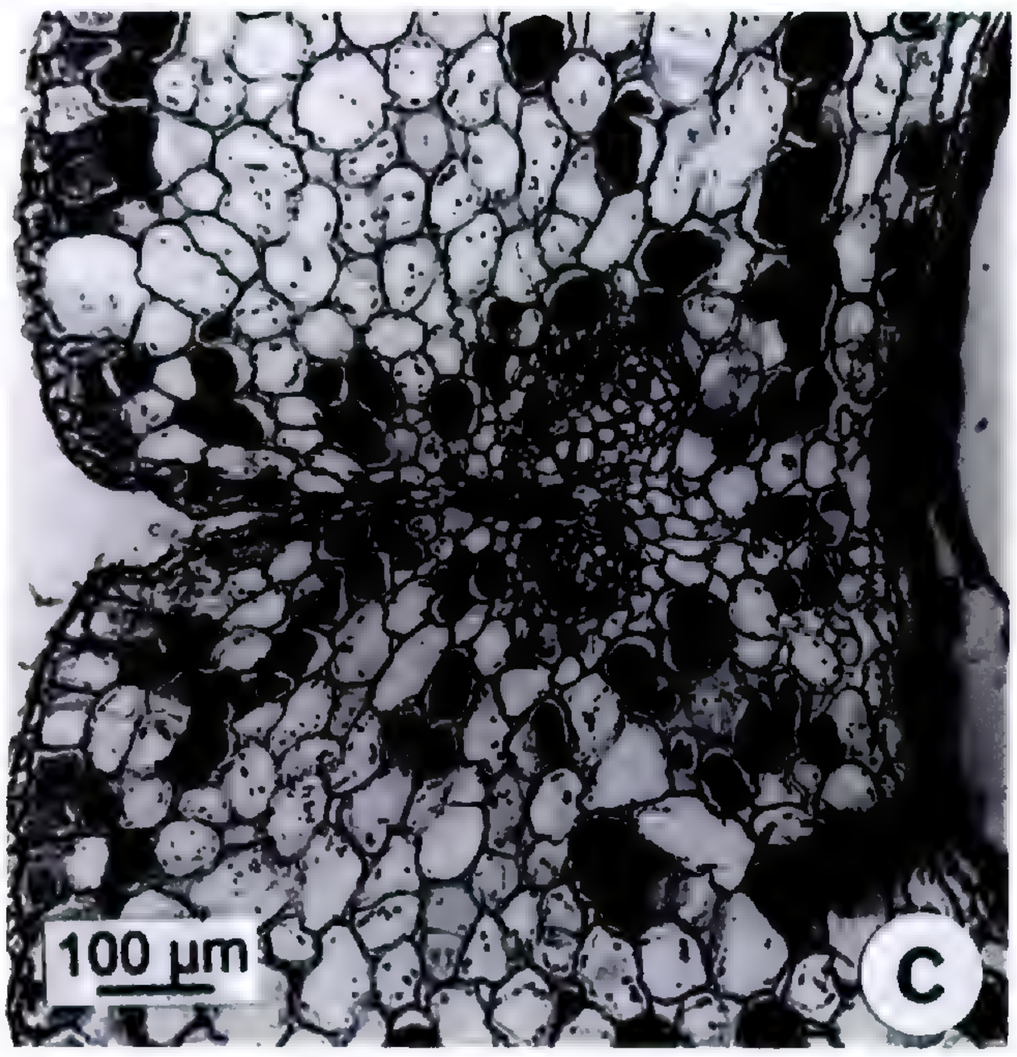
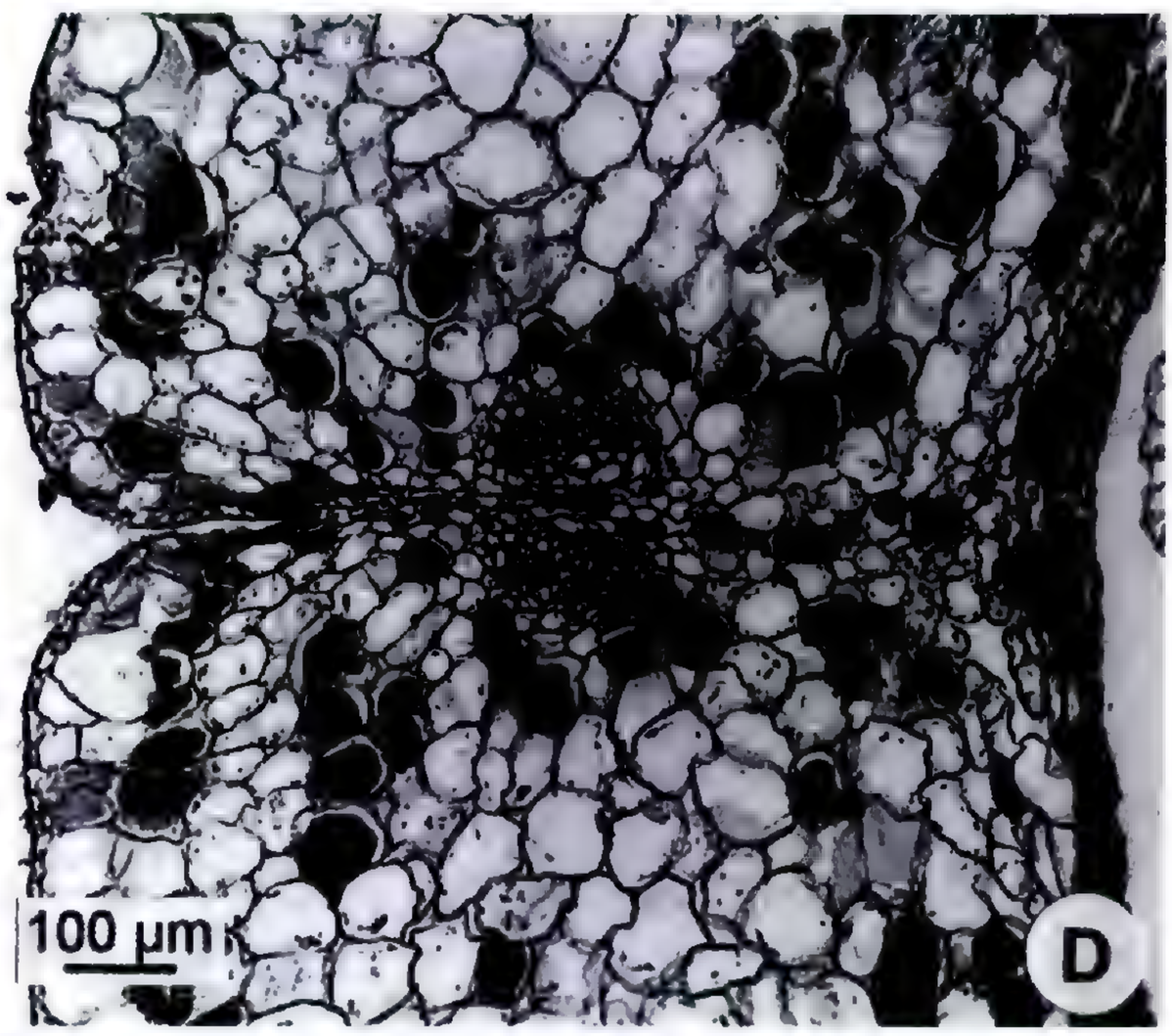


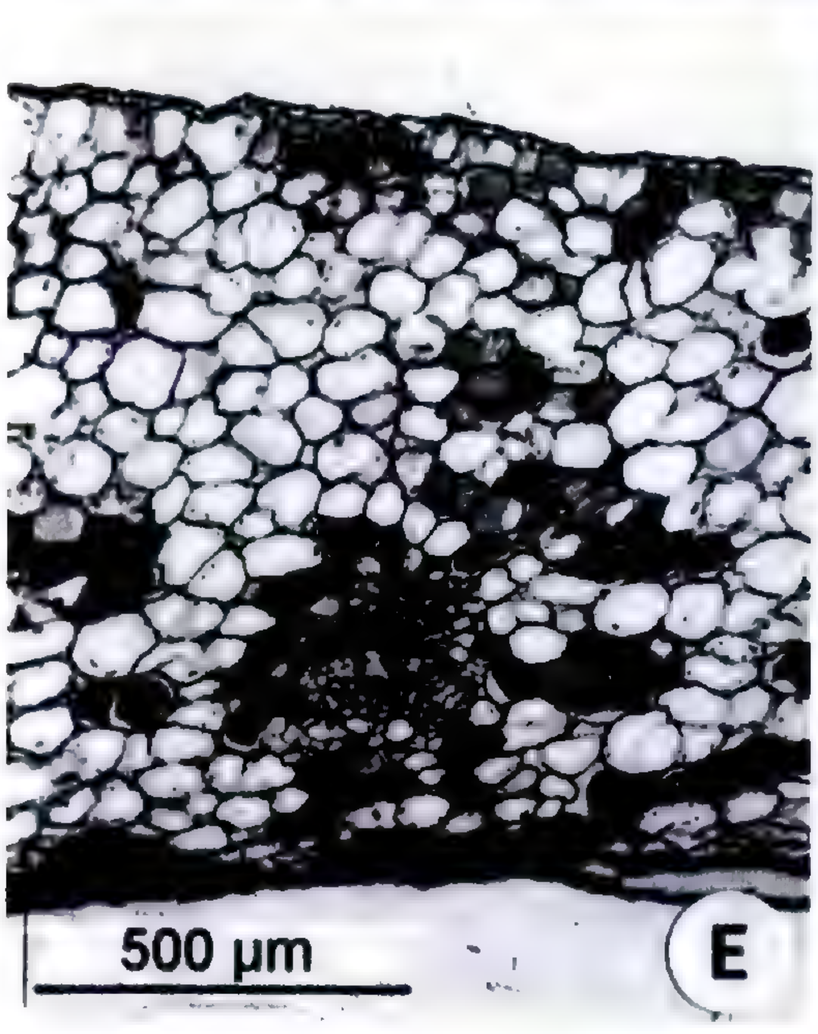
Figure 3. Ascending sections of *Takhtajania* fruit. —A–F. Lower half of the ovary. —G–J. Upper half of the ovary. a1, a2: apical vascular arcs; m: carpellary median; pl: placental bundle; 1, 1′, 2, 2′, 3, 3′, meridian-laterals.











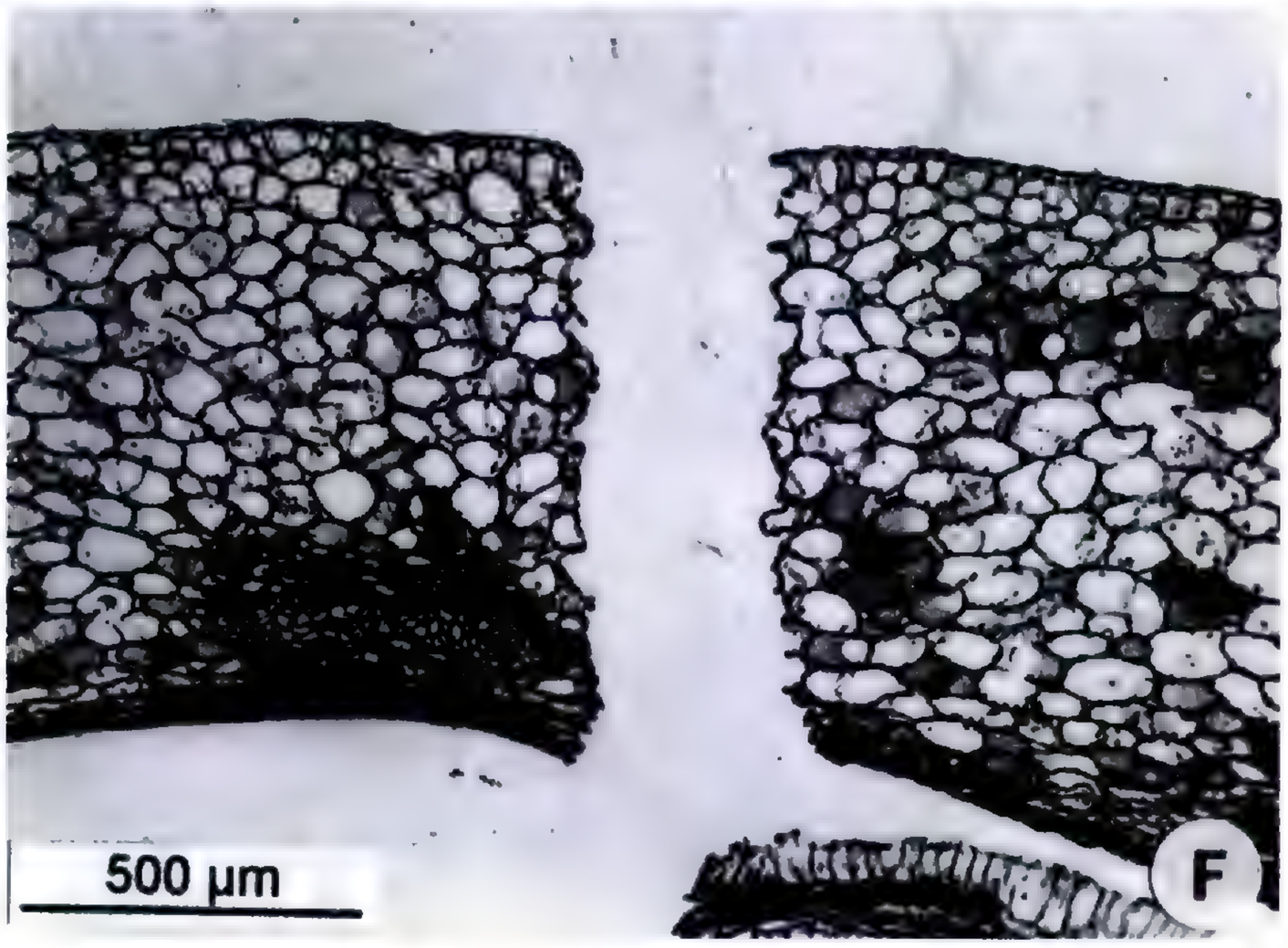


Figure 4. Histology of the fruit wall of *Takhtajania*. —A. Synlateral bundle (sl′) (cf. Fig. 3E). —B. Meridian-lateral bundle 2′ (cf. Fig. 3E above). —C. Transverse section of the wall at the level of the median bundle m1. —D. Transverse section of the wall at the level of the insertion of the seeds. —E. Transverse section of the wall at the level of the meridian-lateral (middle of the ovary locule). —F. Transverse section of the wall at the level of the synlateral (sl) (cf. Fig. 3F); note the unspecialized dehiscence cleft, with no epidermal boundaries.

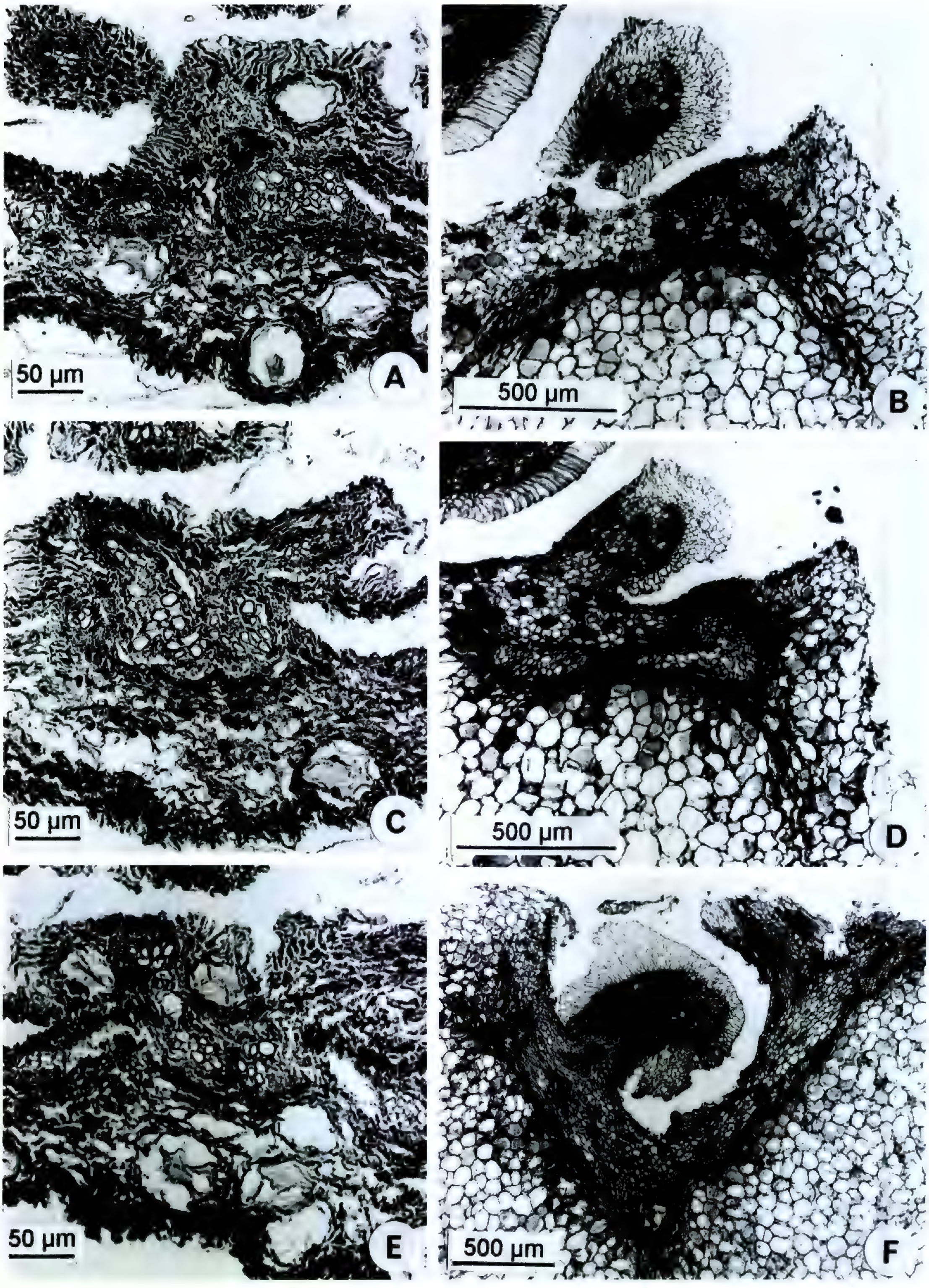


Figure 5. Placental vascularization of *Takhtajania*. —A, C, E. Ascending sections of one of the two placentas at anthesis. —B, D. Ascending sections of half a placenta at fruit stage (cf. 1′, pl 1′, Fig. 3H, I), dehiscence cleft visible on right. —F. Apical vascular arc a2 (cf. Fig. 3, I).

in the wall, and the placental bundles fuse back with the median bundles to form vascular arcs a1 and a2 (Figs. 3I, J, 5F). The vascularization connected to the stigmas naturally disappears along with the latter.

The histological changes of the gynoecium that take place from anthesis to formation of fruit appear to be minor. The ovary wall (Fig. 4E) shows a rather ordinary structure at mid-height: a thin epidermis that is highly fuchsin-stainable throughout the locule and covered with a fairly thick hypodermis that is almost palisadal in places; and lacunar parenchyma ca. 15 cells thick, including some fuchsin-stainable secretory cells as well as cells with calcium oxylate crystals, but lacking sclerified cells. The vascular bundles are surrounded by a crown of highly fuchsin-stainable cells and are largely comprised of metaxylem and metaphloem that are separated by 3 to 5 layers of cambial cells. The protoxylem is crushed and the protophloem is transformed into sclerified fibers with a lumen that is sometimes narrow (Fig. 4A, B). No secondary formations are present in the gynoecium.

At all levels the external epidermis clearly extends to the center of the divided medians (Fig. 4C, D).

Discussion

On the basis of this study, five important features of the fruit of *Takhtajania* can be identified:

(1) Winteraceae appear to be characterized in general by floral vascularization that lacks a cortical system, and in which secondary vascular structures are absent (Nast, 1944; Deroin & Frame, 1998). By contrast, lignification of the xylem and the appearance of phloem fibers are clearly delayed in *Takhtajania*, as compared, for example, to *Exospermum* or *Zygogynum* (Deroin & Frame, 1998), in which differentiation occurs at anthesis. The total absence of sclerenchyma in *Takhtajania*, especially in the fruit wall, appears to be unique within the family, based on the work of Harvey (1982: 164). This type of unspecialized histology is also found in the syncarpous gynoecia of Annonaceae–Monodoroideae (Deroin, 1997), although at anthesis.

(2) The evolution of a stipe, as in most species of *Bubbia* (Winteraceae) (Harvey, 1982: 169), makes it easier to interpret the vascularization at the base of the gynoecium and thus its morphology. In the lower half of the gynoecium the stele divides to form two closed steles (Fig. 2K–P), which in fact clearly represent an incomplete fusion of the peltate bases of the two carpels. This peltation was not visible at anthesis (Deroin & Leroy, 1993) and only appears later in the development of *Takhtajania*, whereas it is rather frequent and probably primitive in other Winteraceae (Leinfellner, 1965, 1966a, 1966b; Endress, 1994; Igersheim & Endress, 1997).

Several very small, isolated bundles are found in the medullary parenchyma (Fig. 2O–P) of the fruit that are not yet present at anthesis, but which are likely homologous with the basal plexus of the gynoecia in Annonaceae and even in Magnoliaceae (Deroin, 1999). Higher up, the steles open once again toward one another while folding back around the median complexes, which divide, as is generally the case in Winteraceae.

(3) From a strictly vascular perspective, the syncarpy found in *Takhtajania* is not particularly advanced, as confirmed by the organization of the stipe and the synlateral bundles, which are often disjointed (Fig. 2U, I1′, and I2′; Fig. 3E, I1, and I2). In this respect the situation is similar to that found in *Monodora brevipes* Benth. (Annonaceae) (Deroin, 1997).

(4) Fruit dehiscence in the material studied is initially difficult to interpret, as the splitting could have resulted from the dehydrating action of the fixative, which would account for the irregularity of the opening. Nevertheless, the simple fact that this phenomenon can occur is of interest, as no other syncarpous member of Magnoliales has dehiscent fruits. Moreover, the possible occurrence of dehiscence in the mericarps of apocarpous Winteraceae is still poorly understood (Harvey, 1982: 167), and was not reported for members of the family in New Caledonia (Vink, 1993).

On the contrary, differentiation of the epidermis between the halves of the divided median bundles suggests primitive dehiscence at this level, which is in fact still functional in many Magnoliaceae and even some archaic Annonaceae such as *Anaxagorea* and *Xylopia*. The presence of apical vascular arcs (Fig. 3I, J, a1, and a2) linking the placental zones, which are present in all Winteraceae studied to date (Bailey & Nast, 1943; Nast, 1944; Ueda, 1978; Harvey, 1982) except *Drimys* (Tucker & Gifford, 1964; Tucker, 1975; Doweld, 1996), does not, however, favor dorsal dehiscence.

(5) The vascular rearrangements during fruit development (Fig. 6) confirm that the nutritive role is transferred from the median carpellary bundle (A) to the lateral bundles (B), a phenomenon that appears to be very generalized among Annonaceae (Deroin, 1997) and Magnoliaceae (Deroin, 1999). The strengthening in the fruit of the meridian-lateral bundles of the first and second orders (Fig. 6,

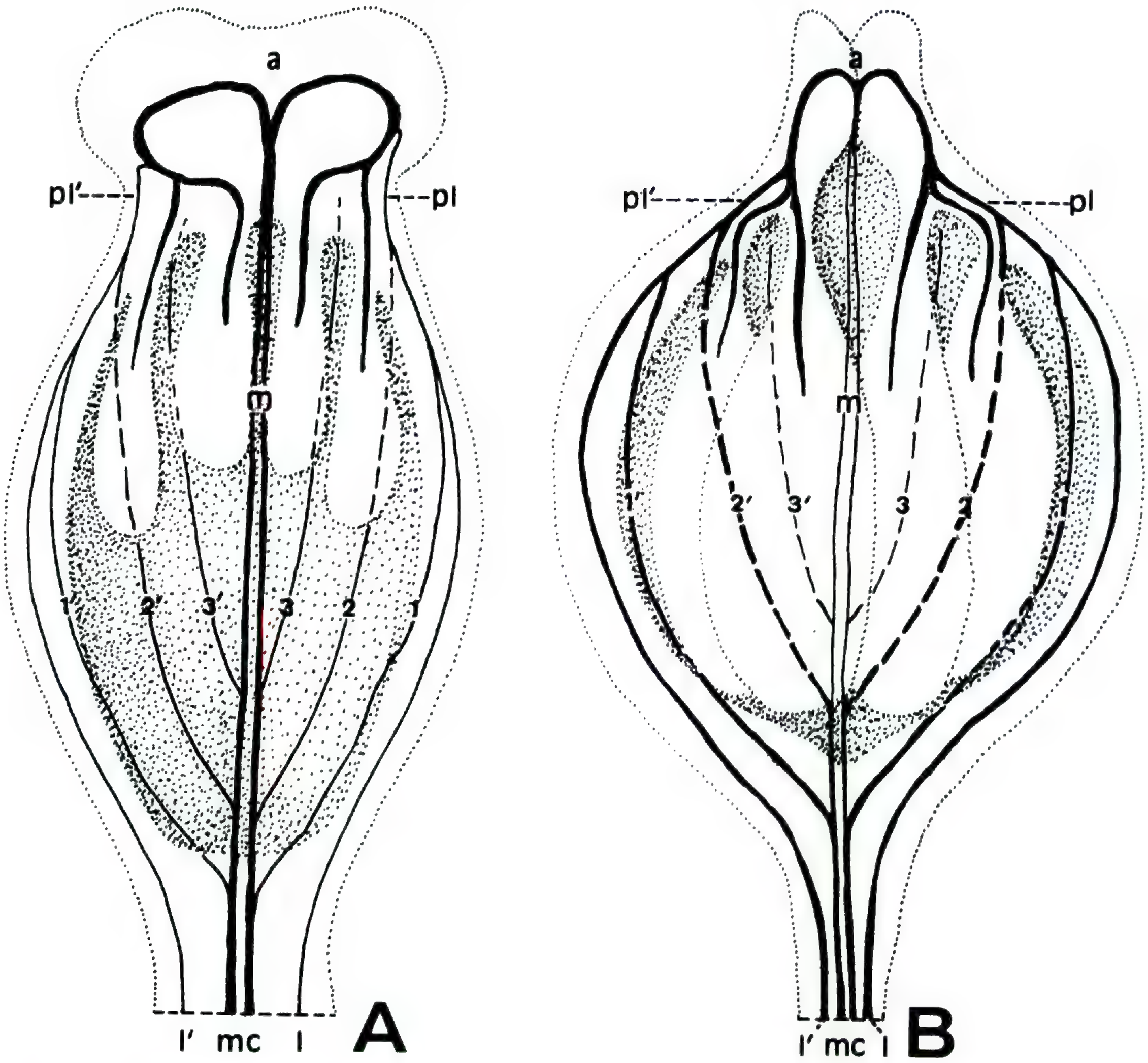


Figure 6. Interpretive schemes of transfer of vascular functionality in the gynoecium of *Takhtajania* from anthesis (A) to mature fruit (B). a: apical vascular arc; l, lateral; m, median; mc, median complex; pl, placental bundle; 1, 2 and 3: median-lateral bundles.

1, 1′, 2, 2′), which then contribute in large part to the apical placental bundles (Fig. 6, pl′, pl), leads to a vascular pattern that is functionally comparable to that of the carpel in *Ambavia gerrardii* (Baill.) Le Thomas, a rather primitive Malagasy Annonaceae, in which the ovules are fed by the meridian-lateral traces (Deroin & Le Thomas, 1989).

In the case of *Takhtajania* (Fig. 6B), eight functional meridian-lateral bundles (four per carpel) feed the eight seeds, with an additional role being played by the synlateral traces. The remaining four meridian-lateral traces, of the third order, most likely represent the relictual vascularizations of four additional ovules. While the closely related genus *Bubbia* has an average of 12 ovules per carpel (Harvey, 1982: 172), the genus *Takhtajania* appears to have undergone a reduction in the number of ovules (to 4 per carpel in the material studied), perhaps following fusion of the carpels. This reduction does not appear to have occurred in the syncarps of Annonaceae–Monodoroideae (Deroin, 1991), and instead results from the distinctive placentation of Winteraceae.

Conclusions

Study of the fruit of *Takhtajania* shows that only modest changes have taken place in the organization of the ovary, but that they are nevertheless very instructive. The latent peltation of the carpels and the formation of a very short stipe resemble the tricarpellate apocarpous gynoecium of *Anaxagorea luzonensis* A. Gray, a primitive Annonaceae (Deroin, 1988), and thus suggest the early appearance

of syncarpy as in Annonaceae–Monodoroideae (Deroin, 1991), which preserves in some way the primitively open carpel structure. The vascularization of the seeds shows a delayed pattern which parallels that of the ovules of the syncarpous gynoecium of Canellaceae (Leinfellner, 1967); the gynoecium of *Takhtajania* is thus paedomorpic, with a carpel pattern very near that of *Bubbia*, a rather few-carpelled and thus archaic genus (1 to 11 carpels after Harvey, 1982: 148). In these two genera the dehiscence was at first dorsal, as demonstrated by the epidermal differentiation at the median trace level, the ventral dehiscence being restored in *Takhtajania* because of the imperfection in vascular syncarpy. By comparison, in Annonaceae–Monodoroideae, all with indehiscent fruits, however, vascular syncarpy is yet more advanced in *Isolona* than in *Monodora*, sometimes even with an ultimate reduction and disappearance of synlateral traces (Deroin, 1997). When considering the continuum from the flower to the fruit, this morphological and functional convergence among Gondwanan Magnoliales appears to highlight their shared dispersal syndrome, likely due to common paleoenvironmental constraints.

As a final remark, it is noticeable that the main vascular rearrangements during the fruit set are located in the winteraceous gynoecium only, while the regular receptacle vasculature too is subject to disruptions in Magnoliaceae and Annonaceae, in close relation with seed nutrition (Deroin, 1999).

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CHROMOSOMES OF *TAKHTAJANIA*, OTHER WINTERACEAE, AND CANELLACEAE: PHYLOGENETIC IMPLICATIONS[1]

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Abstract

The somatic chromosome number of *Takhtajania perrieri*, the only relict representative of Winteraceae on Madagascar, is documented as $2n = 36$. The possibly paleotetraploid karyotype appears "diploidized." Chromosome size and behavior, as well as structure of interphase nuclei, correspond to the prochromosome type, widespread among the Magnoliid assemblage. Chromosome numbers available for all other Winteraceae and the related Canellaceae (Winterales) are critically compiled, compared, and interpreted. In light of the postulated molecular phylogeny of the two families, it appears that several successive phases of polyploidization and parallel descending dysploid series coupled with much extinction can explain the karyological features and distribution of the few surviving taxa.

Key words: Canellaceae, chromosome number, diploidization, evolution, paleopolyploidy, *Takhtajania*, Winteraceae.

Takhtajania perrieri (Capuron) Baranova & J.-F. Leroy was discovered in 1909 by Perrier de la Bâthie in the Massif du Manongarivo of northwestern Madagascar, where the population is now extirpated. Only in 1963 was it described as *Bubbia perrieri* by Capuron. After 85 years, its rediscovery in another locality of northwestern Madagascar, 150 km to the east, in the Anjanaharibe-Sud Special Reserve (Schatz et al., 1998) has made it possible to collect material for a first karyological study of this fascinating relict plant. After several failures with fixations of untreated roots and flower buds (smallest about 2 mm diam.), already too advanced for the study of microsporocyte meioses or pollen mitoses, preliminary results were finally obtained on mitotic chromosome number and morphology, as well as interphase nuclear structure from pretreated root tips.

This breakthrough has stimulated a critical compilation of all chromosome counts (and additional karyological observations) published so far on the families of Winteraceae and Canellaceae. That these families are related and somewhat isolated as Winterales within the Magnoliid assemblage (sister to Piperales sensu lato) has been well supported recently by plastid, mitochondrial, and nuclear DNA sequences (Soltis et al., 1999; Qiu et al., 1999). The aim of the present report is to summarize the still quite incomplete karyological data on all taxa of Winterales (Table 1) and to compare them with available information from DNA data as well as other evidence (see Vink, 1970, 1985, 1993; Kubitzki, 1993). Thus, we hope to contribute to a more unified picture of the evolution and phylogenetic history of the two Winterales families.

Materials and Methods

The material used for our study was collected by C. Birkinshaw, 7–11 September 1997, at the Anjanaharibe-Sud Special Reserve, 14°45′S, 49°29′E, at an altitude of 1100–1300 m. Juvenile plants less than 20 cm tall but exhibiting adult leaf morphology were observed growing adjacent to reproductive adults. Ten of these were transplanted into containers and transported to Antananarivo. This population is represented by the fruiting voucher *C. Birkinshaw et al. 483*. At the time that root tips were fixed for the present study (June 1998) only two of the ten plants remained alive; both of these have subsequently died.

Root tips were pretreated in a 0.05% solution of colchicine for 3.5 hours at room temperature, fixed in a 1:3 mixture of glacial acetic acid and 96% ethanol, and stored in a deep-freezer until required.

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Table 1. Critical survey of chromosome number records available for Winteraceae and Canellaceae. First column with currently accepted and valid names of taxa, second column with names used in the original karyological publications.

Taxon	Published name	Provenance	*n*/2*n*	Reference
Winteraceae				
Takhtajania perrieri (Capuron) Baranova & J.-F. Leroy	same	Madagascar, Anjanaharibe-Sud	$2n = 36$	this publication
Tasmannia insipida R. Br. ex DC.	*Drimys insipida* (R. Br.) Pilger	Australia, N. S. Wales	$n = 13$	Hotchkiss (1955)
T. insipida R. Br. ex DC.	*Drimys insipida* (R. Br.) Pilger	Australia, N. S. Wales	$n = 13$, $2n = 26$	Ehrendorfer et al. (1968)
T. insipida R. Br. ex DC.	*Drimys insipida* (R. Br.) Pilger	Australia, S. Queensland	$n = 13$, $2n = 26$	Ehrendorfer et al. (1968)
T. lanceolata (Poir.) A. C. Smith	*Drimys lanceolata* (Poir.) Baill.	Australia, N. S. Wales	$2n = 13$	Hotchkiss (1955)
T. lanceolata (Poir.) A. C. Smith	*Drimys lanceolata* (Poir.) Baill.	? Australia	$2n = 26$	Smith-White in Darlington & Wylie (1955)
T. lanceolata (Poir.) A. C. Smith	*Drimys lanceolata* (Poir.) Baill.	? cult.	$2n = 28$[1]	Gulline in Darlington & Wylie (1955)
T. lanceolata (Poir.) A. C. Smith	*Drimys aromatica*	? cult.	$2n = 38$[2]	E K. Janaki Ammal in Darlington & Wylie (1955)
T. lanceolata (Poir.) A. C. Smith	*Drimys lanceolata* (Poir.) Baill.	? cult.	$2n = 26$	R. Gankin in Stebbins (1958)
T. lanceolata (Poir.) A. C. Smith	*Drimys lanceolata* (Poir.) Baill.	cult.	$2n = 26$	Raven & Kyhos (1965)
T. lanceolata (Poir.) A. C. Smith	*Drimys lanceolata* (Poir.) Baill.	Australia, N. S. Wales	$n = 13$, $2n = 26$	Ehrendorfer et al. (1968)
T. lanceolata (Poir.) A. C. Smith	*Drimys lanceolata* (Poir.) Baill.	cult.	$2n = 26$	Ratter & Milne (1973)
T. piperita (Hook f.) Miers "entity 25. *heteromera*" (Vink, 1970)	*Drimys hatamensis* Becc.	Papua-New Guinea, Mt. Wilhelm	$2n = 28$[1]	Borgmann (1964)
T. piperita (Hook f.) Miers "entity 32. *cordata*" (Vink, 1970)	*Drimys rosea*	Papua-New Guinea, Mt. Wilhelm	$2n = 28$[1]	Borgmann (1964)
T. piperita (Hook f.) Miers "entity 38. *membranea*" (Vink, 1970)	*Drimys membranea* F. v. Muell.	Australia, N. Queensland	$n = 13$, $2n = 26$	Ehrendorfer et al. (1968)
T. piperita (Hook f.) Miers	*Drimys* sect. *Tasmannia*, "one polymorphic New Guinea species"	Papua-New Guinea, Wau	$n = 13$	Ehrendorfer et al. (1968)

Table 1. Continued.

Taxon	Published name	Provenance	*n*/2*n*	Reference
T. piperita (Hook f.) Miers	*Drimys piperita* Hook f.	Papua-New Guinea (two individuals)	a: $n = 13$, b: $2n = 52$[3]	Ratter & Milne (1973)
T. purpurascens (Vickery) A. C. Smith	*Drimys purpurascens* Vickery	Australia, N. S. Wales	$n = 13$	Hotchkiss (1955)
T. stipitata (Vickery) A. C. Smith	*Drimys stipitata* Vickery	Australia, N. S. Wales	$n = 13$	Hotchkiss (1955)
Drimys *brasiliensis* Miers subsp. *brasiliensis*	same	Brazil, São Paulo	$2n =$ ca. 86	Ehrendorfer, Silberbauer-Gottsberger & Gottsberger (1979)
D. brasiliensis Miers subsp. *sylvatica* (St. Hil.) Ehrend. & Gottsb.	same	Brazil, Rio de Janeiro	$2n = 86$	Morawetz (1984)
D. confertifolia Phil.	same	Juan Fernández Isl.	$n =$ ca. 43	Sun et al. (1990)
D. granadensis L.f. var. *mexicana* A. C. Sm.	same	Costa Rica	$n = \pm 43$, $2n = \pm 86$	Ehrendorfer et al. (1968)
D. winteri J. R. & G. Forst.[4]	same	cult.	$n =$ ca. 36[5], $2n =$ ca. 72[5]	Strasburger (1905)
D. winteri J. R. & G. Forst.[4]	same	cult.	$2n = \pm 76$[5]	Whitaker (1933)
D. winteri J. R. & G. Forst.[4]	same	cult. (two prov.)	$n = 43$[6]	Raven & Kyhos (1965)
D. winteri J. R. & G. Forst.[4]	same	cult.	$n = 43$	Ratter & Milne (1973)
Pseudowintera *axillaris* (J. R. & G. Forst.) Dandy	same	New Zealand, Wellington	$n = 43$	Hair in Raven & Kyhos (1965), Hair & Beuzenberg (1966)
P. colorata (Raoul) Dandy	same	New Zealand, cult.	$n = 43$	Hair in Raven & Kyhos (1965), Hair & Beuzenberg (1966)
P. traversii (Buchanan) Dandy	same	New Zealand, NW Nelson	$n = 43$	Hair & Beuzenberg (1966)
Bubbia[7] *semecarpoides* (F. v. Muell.) Burtt	same	Australia, N. Queensland (two populations)	a: $2n = 86$ (–88) b: $2n = \pm 86$	Ehrendorfer et al. (1968)
B. spec.	same	Papua-New Guinea, Wau	$2n =$ (84)–86	Ehrendorfer et al. (1968)
Belliolum[7] *crassifolium* (Baill.) v. Tiegh.	same	New Caledonia	$2n =$ ca. 86	Ehrendorfer et al. (1968)
Zygogynum[7] *baillonii* v. Tiegh. (vel aff.)	same	New Caledonia	$n = 86$, $2n = 172$	Ehrendorfer et al. (1968)
Z. balansae v. Tiegh	*Zygogynum pomiferum* Baill. subsp. *balansae* (v. Tiegh.) Vink	New Caledonia	$n =$ ca. 43	Carr & Mc Pherson (1986)

Table 1. Continued.

Taxon	Published name	Provenance	*n*/2*n*	Reference
Canellaceae				
Canella *winteriana* (L.) Gaertn.	*Canella alba* Murr.	USA, Florida	$2n = 28$	Goldblatt in Raven (1975), Goldblatt (1977)
Cinnamodendron *axillare* (Nees & Mart.) Endl.	same	Brazil, Rio de Janeiro	$2n = 22$	Occhioni (1948)
Capsicodendron *dinisii* (Schw.) Occhioni[8]	*Capsicodendron Dinissii*	Brazil, cult.	$2n = 26$	Occhioni (1945)

[1] Obviously erroneous for $2n = 26$ (see Raven & Kyhos, 1965).
[2] Possibly a triploid plant (Raven & Kyhos, 1965) or rather a wrong determination; no voucher!
[3] Obviously an autotetraploid plant.
[4] In view of the former wide circumscription of *Drimys winteri* (now limited to populations from central and southern Chile and adjacent Argentina), the exact determination of cultivated material remains doubtful
[5] Obviously erroneous for $n = 43$ or $2n = 86$, respectively.
[6] Partly with one additional fragment.
[7] The genera *Bubbia*, *Belliolum*, *Exospermum*, and *Zygogynum* s. str. are united by Vink (1985, 1993) under *Zygogynum* sensu lato.
[8] To be incorporated into *Cinnamodendron* (Kubitzki, 1993)?

Chromosome preparation by the air-drying method was performed according to Geber and Schweizer (1988), except that the root apices were softened in an enzymatic solution (2% cellulase plus 20% pectinase) at 37°C for 3 hours. The slides were stained in a DAPI (4′-6-diamidino -2 phenylindole) solution of 2 μg/ml in Mc Ilvain's buffer pH7 for 20 minutes and mounted in Mc Ilvain's buffer pH7 plus glycerol 2:1 (see Schweizer & Ambros, 1994). Chromosome fluorescence was observed with a Leizt Orthoplan fluorescence microscope with filter block A for DAPI epiluminescence. Microphotographs were taken on Kodak TMAX 400 film.

Results

Well spread earlier and later mitoses and interphase nuclei from pretreated root tips of *Takhtajania perrieri* are shown in Figure 1. The nuclei belong to the prochromosome type: Their intensively stained elements correspond to the proximal and evidently heterochromatic segments of the chromosomes, which remain ± condensed during interphase and form about 36 larger and smaller chromocenters (Fig. 1b, c). These chromocenters exhibit a certain tendency toward agglomeration (particularly obvious in those of larger size) and are embedded in a very weakly stained granular-fibrous matrix. During mitotic prophase and early metaphase condensation of chromosome segments in a proximal → distal direction becomes apparent with the distal regions first visible as fibrous attachments (Fig. 1a). This process is concluded toward the end of metaphase (Fig. 1b), just before the onset of anaphase.

From the careful inspection of about 20 mitotic plates the somatic chromosome number $2n = 36$ can be ascertained. These chromosomes are of quite different sizes, the largest ones (about 4 to 5 pairs) reach a length of 2.0–2.5 μm in our pretreated material, the smallest (one pair), not always easily recognizable (see arrow heads in Fig. 1a and b), about 0.8 μm. We are not yet able to present a karyogram for *Takhtajania*, but there are certainly not four structurally ± identical chromosome sets and therefore no clear karyological indications for tetraploidy.

Discussion

The interphase nuclei, chromosome size, and mitotic condensation pattern found in *Takhtajania perrieri* correspond well with the prochromosome type described by Tanaka (1971), Okada (1975: type C), and Morawetz (1986a: Gyrocarpus type) for many Magnolid orders such as Magnoliales (Magnoliaceae, Annonaceae, etc.) and Laurales (Lauraceae, Calycanthaceae, etc.). Within Winteraceae and Win-

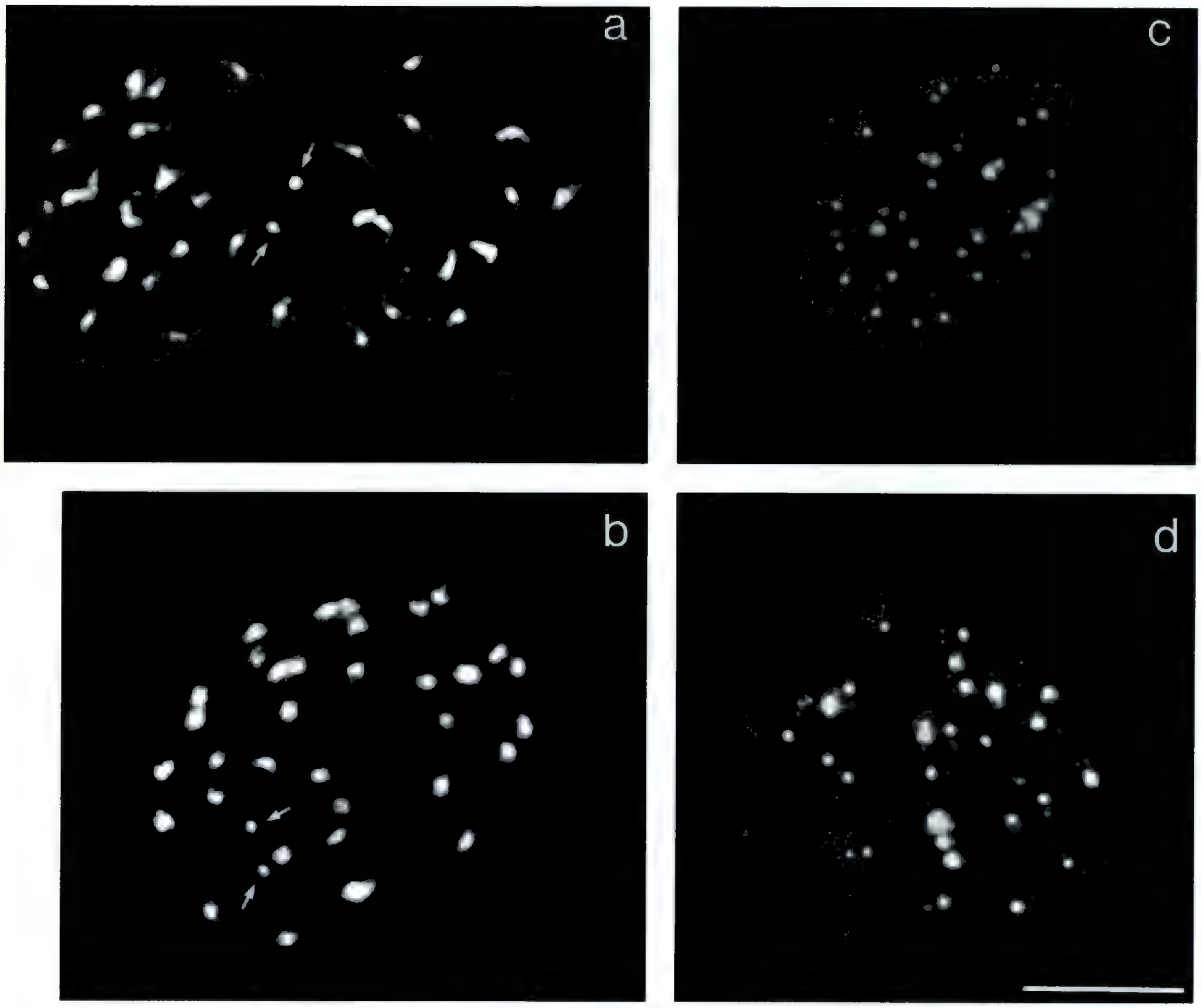

Figure 1. DAPI stained chromosomes (a, b) and interphase nuclei (c, d) of *Takhtajania perrieri* from pretreated root tips. In early metaphase (a) chromosomes fully condensed only in proximal segments, distally still fibrous, in late metaphase (b) completely condensed. Arrows mark the pair of smallest chromosomes. Interphase nuclei (c, d) are of the prochromosome type. The bar corresponds to 10 μm.

terales comparable karyological data are still limited, but *Drimys* s. str., in spite of its much higher chromosome number, exhibits quite similar karyological features to *Takhtajania* (Morawetz, 1984). Thus, there is no inconsistency in this respect to the placement of *Takhtajania* into the Winteraceae, and of Winterales, together with Magnoliales and Laurales, into the Magnoliid assemblage. Nevertheless, these karyological features may vary quite considerably within orders and families (e.g., *Cassytha* within Laurales: Okada, 1975; *Uvariopsis* and *Tetrameranthus* within Annonaceae: Morawetz, 1986a, b, 1988). This also applies to Winteraceae because preliminary and unpublished observations indicate that *Tasmannia* not only deviates from all other members of the family by its low chromosome number ($x = 13$), but also by much larger chromosomes and interphase nuclei that correspond rather to type B of Okada (1975). Together with the molecular evidence (Suh et al., 1993; Karol et al., 2000 this issue; Fig. 2) this argues strongly against the combining of *Tasmannia* and *Drimys* into a single genus as advocated by Vink (1970, 1988, 1993). Nevertheless, much more detailed and comparative studies on karyotypes, banding patterns, and DNA quantities in the members of Winterales and other Magnoliids are necessary before we can better understand the karyoevolution of these basal Angiosperms.

What are the phylogenetic implications of the chromosome number $2n = 36$ established for *Takhtajania* relative to other members of Winterales? The main evidence compiled in Table 1 is compared in Figure 2 with our present knowledge about the evolutionary divergence of the order. This shows that from four extant clades three (1–3) have remained on a low ploidy level: (1) *Takhtajania* ($n = 18$) and (2) *Tasmannia* ($n = 13$) within Winteraceae, and (3) Canellaceae ($n = 14, 13, 11$). Clade

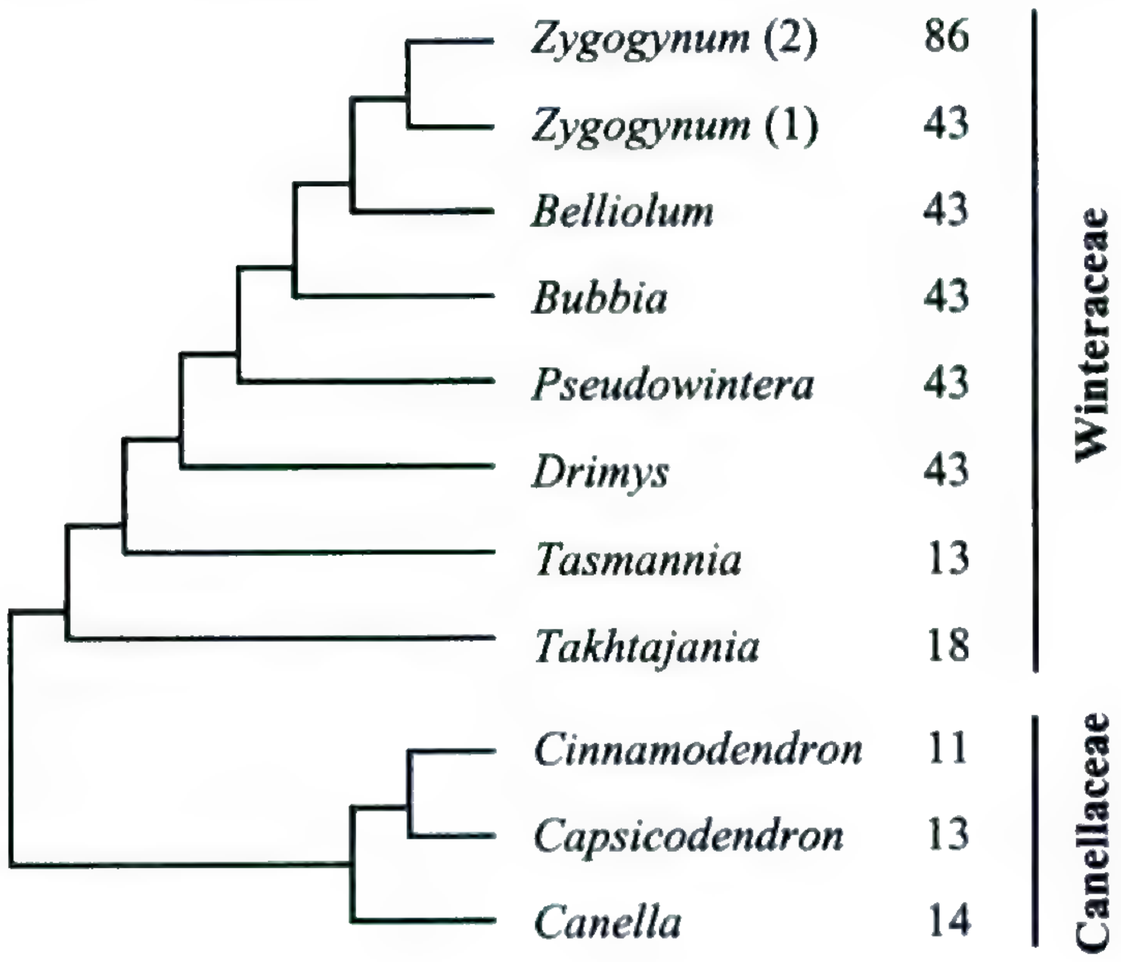

Figure 2. Molecular tree (ITS and *trn*L) of Winteraceae and Canellaceae, adapted from Karol et al. (2000) with genera for which chromosome numbers are known; haploid numbers (*n*) inserted. *Zygogynum* (1) = *Z. baillonii*, (2) = *Z. balansae*. See Table 1.

(4) has become stabilized on a higher polyploid level ($n = 43$, rarely with additional polyploidy: $n = 86$) and includes all remaining Winteraceae, i.e., *Drimys*, *Pseudowintera*, and the *Zygogynum* s.l. group of closely related genera. It is obvious that considerable evolutionary changes and numerous extinction events during the well documented and extremely long geological history of Winterales (since the Lower Cretaceous: see Doyle, 2000 this issue) must have resulted in the present-day highly relictual pattern of the Winterales.

There is some evidence that polyploids have become diploidized by considerable restructuring of their karyotypes (*Drimys*: Morawetz, 1984; *Takhtajania*: see above) and now appear as "paleopolyploids." In addition, chromosomal rearrangements must have led to dysploid and/or Robertsonian changes of base numbers at different ploidy levels. In Annonaceae and Eupomatiaceae this pattern of chromosomal evolution is still better preserved and more easily interpreted (Morawetz 1986a: fig. 16; 1988). It exhibits dysploid differentiation on the diploid level with extant taxa on $n = 7 \leftarrow 8 \rightarrow 9 \rightarrow 10$ as well as $4x$, $6x$, and higher polyploid series on each of these base numbers. This pattern can serve as a model for the evolutionarily much more "eroded" Winterales where such a reconstruction suffers from many extinction gaps. Nevertheless, Figure 3 illustrates possible links between chromosome numbers of extant representatives (Grant, 1982).

According to the ITS and *trn*L tree (Suh et al., 1993; Karol et al., 2000), *Takhtajania* with $n = 18$ and *Canella* with $n = 14$ are the most basal branches of Winteraceae and Canellaceae, respectively. If paleotetraploidy is postulated for these two extant relicts, then $n = 13$ in *Tasmannia* (Winteraceae) as well as $n = 13$ and $n = 11$ in the remaining Canellaceae could have originated by descending dysploidy. The origin of the highly polyploid Winteraceae clade (4) with $n = 43$ (paleo-$12x$?) was evidently linked with a remarkable duplication event in the ITS region (Suh et al., 1993; Karol et al., 2000). The present diversity center of this clade is in Australasia, i.e., former eastern Gondwanaland, from where it could have reached New Zealand with *Pseudowintera* and also South America via Antarctica (?) with *Drimys*.

In retrospect, Winterales correspond in their evolutionary pattern to other tropical, woody and "basal" Angiosperm groups (Ehrendorfer, 1976, 1987, 1995). They exhibit features of stasigenesis (evolutionary "erosion," much extinction), but also show signs of very active and recent eco-geographical radiation (e.g., in *Drimys*: Ehrendorfer et al., 1979; *Tasmannia*: Vink, 1970).

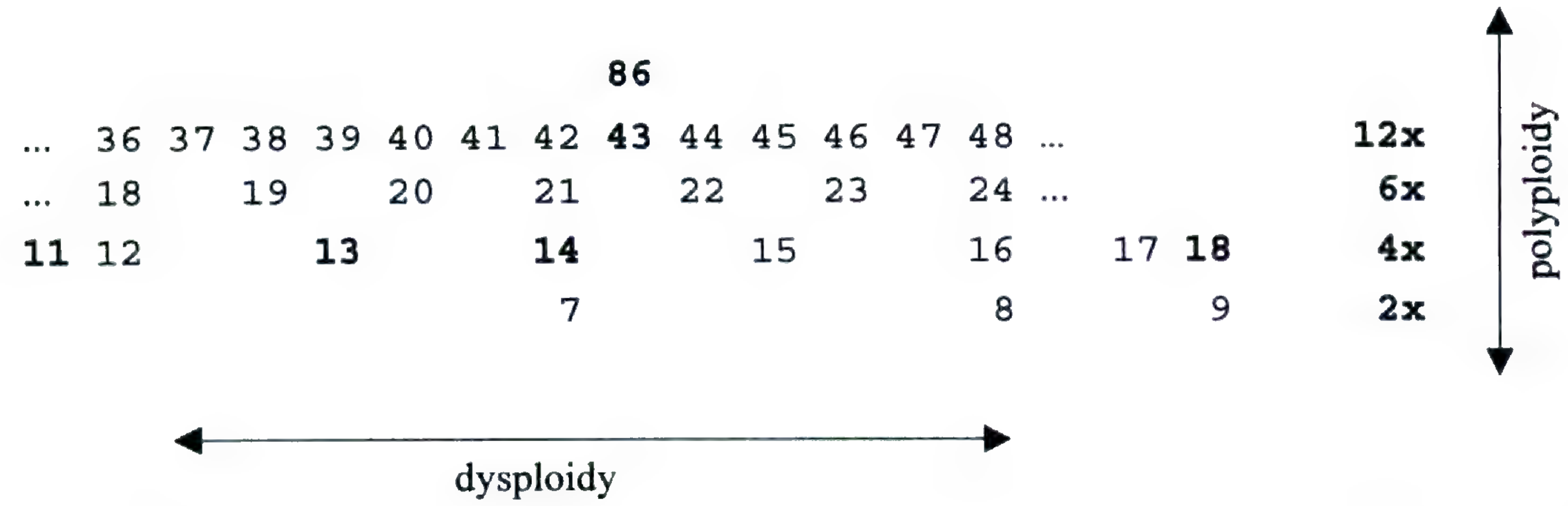

Figure 3. Possible origins of haploid chromosome numbers (*n*) of extant Winteraceae and Canellaceae (in bold print: 11, 13, 14, 18, 43, 86) as a result of polyploid and dysploid changes in hypothetical ancestral taxa (chromosome numbers in regular print).

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MOLECULAR EVIDENCE FOR THE PHYLOGENETIC POSITION OF *TAKHTAJANIA* IN THE WINTERACEAE: INFERENCE FROM NUCLEAR RIBOSOMAL AND CHLOROPLAST GENE SPACER SEQUENCES[1]

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Abstract

The nucleotide sequences of the internal transcribed spacers (ITS 1 and ITS 2) and 5.8S coding region of nuclear ribosomal DNA, as well as the non-coding *trn*L-*trn*F spacer regions of the chloroplast DNA, were determined and analyzed to estimate the phylogenetic position of *Takhtajania perrieri* (Capuron) Baranova & J.-F. Leroy within the Winteraceae. Using representatives of each genus of Canellaceae as outgroups (*Canella, Capsicodendron, Cinnamodendron, Cinnamosma, Pleodendron,* and *Warburgia*), both maximum parsimony and maximum likelihood analyses place *Takhtajania* in a basal position sister to the remainder of the Winteraceae. Although the overall topology within the Winteraceae was mostly congruent between nuclear and chloroplast data sets, the *trn*L-*trn*F data resulted in lower support values in comparison to the ITS data, and failed to resolve basal relationships in the family, yielding alternative equally parsimonious solutions. The combined nuclear/chloroplast data set resulted in a single tree identical to that generated by nuclear data alone, but demonstrated strengthened support for a basal branch leading to *Takhtajania,* as well as for the position of *Tasmannia* as the next branch within the family. Potentially conflicting signals from nuclear and chloroplast data indicate that further taxon sampling or additional sequence data may be required to infer infrafamilial phylogenetic relationships for Canellaceae.

Key words: Canellaceae, combined data, cpDNA, ITS, Likelihood, phylogeny, *Takhtajania,* Winteraceae.

Known until recently from only a single collection made in 1909, *Takhtajania perrieri* has long been an enigma among the basal angiosperm family Winteraceae (Leroy, 1978). Its rediscovery in 1994 (Schatz et al., 1998) has provided the opportunity to reevaluate those morphological features anomalous within the family, as well as to explore the phylogenetic relationship of this isolated, relictual Malagasy endemic. Based upon morphological characters, two alternative hypotheses have been proposed regarding the position of *Takhtajania* within the Winteraceae: (1) *Takhtajania* is a member of a clade also comprising *Pseudowintera, Belliolum, Bubbia, Exospermum,* and *Zygogynum* (the latter 4 genera sometimes combined into a single genus *Zygogynum* s.l.) (Vink, 1988; Endress et al., 2000 this issue); or (2) *Takhtajania* represents the sister taxon to the remainder of the Winteraceae (Leroy, 1978; Vink, 1988). The aim of the present study is to infer the phylogenetic position of *Takhtajania* based upon analyses of molecular sequence data.

A previous molecular phylogenetic study (Suh et al., 1993) utilized the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (nrDNA) to address questions of generic relationships in the Winteraceae. Tissue of *Takhtajania,* however, was unavailable. In addition to presenting a phylogeny

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for the family solely through ingroup analysis, Suh et al. (1993) identified a single gene duplication event in the *Zygogynum* s.l. clade, a possible molecular marker for the inclusion (or exclusion) of *Takhtajania* within the *Belliolum/Bubbia/Exospermum/Zygogynum* assemblage.

If an ITS duplication event either was not identified in *Takhtajania* or occurred independently, then outgroup analysis would become critical for investigating its relationship to the remainder of the family. Both morphological and molecular (*rbc*L) cladistic studies, which examined relationships among basal angiosperms, have identified Canellaceae (represented in these studies by *Canella* alone) as a putative sister taxon to Winteraceae (Donoghue & Doyle, 1989; Qiu et al., 1993; Chase et al., 1993). *Canella* also appeared to be closely related to Winteraceae on the basis of secondary metabolites (Gottlieb et al., 1989). Canellaceae are comprised of six genera found in tropical Africa (*Warburgia*), Madagascar (*Cinnamosma*), and tropical America (*Capsicodendron* and *Cinnamodendron* in South America, *Canella* and *Pleodendron* in the Caribbean) (Cronquist, 1981). Although Suh et al. (1993) had encountered difficulties in the alignment of ITS sequences between *Canella* and Winteraceae, it was decided to reattempt alignment after sequencing additional representatives of Canellaceae.

This paper reports on the implications of new ITS sequence data for *Takhtajania* and the five other genera of Canellaceae; *Canella* ITS sequence had been previously determined by Suh et al. (1992). In addition, sequence data have been generated for two tandem chloroplast encoded (cpDNA) spacer regions, and analyzed both separately and in combination with the ITS sequences. The first spacer is a group I intron and is located between the conserved *trn*L (UAA) 3′ exon and *trn*L (UAA) 5′ exon. The second, an intergenic spacer, is located between the *trn*L (UAA) 5′ exon and *trn*F (GAA) (Taberlet et al., 1991). Restriction fragment length polymorphisms of this molecule have been used to address species-level relationships within Mexican pines (Perez de la Rosa et al., 1995). Sequence data generated from the *trn*L-F region also have been utilized to resolve phylogenetic relationships at both the species level (Mes & Hart, 1994) and the generic level (Compton et al., 1998; McDade & Moody, 1999). Several studies (Gielly et al., 1996; Molvray et al., 1999) have demonstrated the utility of combining the ITS and *trn*L-F spacer sequences for phylogenetic reconstruction. Thus, a complete survey of both ITS and *trn*L-F regions for all genera of both Winteraceae and Canellaceae has been conducted to estimate the phylogenetic position of *Takhtajania*.

Materials and Methods

Species Sampled and Sources of Plant Material

The species included in this study are presented in Table 1, along with voucher information, literature citation, and GenBank accession numbers for both the ITS and *trn*L-F spacer sequences. This list includes at least one representative species from all genera in both the Winteraceae and Canellaceae. In the instances where ITS has been determined previously (all Winteraceae [Suh et al., 1993] except *Takhtajania*, plus *Canella* [Suh et al., 1992]), identical DNA extracts were used to amplify and sequence the *trn*L-F spacers.

DNA Extraction, Amplification, and Sequencing

Standard CTAB methods of DNA isolation (Doyle & Doyle, 1987) were used to obtain total genomic DNA for polymerase chain reaction (PCR) amplification. To generate ITS amplicons, PCR was performed using plant specific primer ITS5 (Suh et al., 1993) and universal primer ITS4 (White et al., 1990) under the conditions described in Suh et al. (1993). To generate the *trn*L-F spacer amplicons, PCR was performed using universal primers "c" and "f" (Taberlet et al., 1991), also under the conditions described in Suh et al. (1993).

Sequence data were generated from PEG (polyethylene glycol) purified double-stranded PCR products (Morgan & Soltis, 1993) with the dye-terminator cycle-sequencing protocol for the ABI 373A Sequencer (Applied Biosystems, Inc.). The entire forward and reverse strand of ITS was determined for each species using the amplification primers (see above) and primers ITS3 (White et al., 1990) and C5.8S (Suh et al., 1993). To determine the sequence for both strands of the *trn*L-F spacers, the amplification primers were used as well as primers "d" and "e" (Taberlet et al., 1991). The resulting chromatograms were edited with Sequencher version 3.1 (Gene Codes, Inc.); regions corresponding to the amplification primers were deleted. The final edited consensus sequences were exported for alignment.

Sequence Alignment

All sequences were aligned manually with the aid of Se-Al version 1.0a1 (Rambaut, 1996) multiple sequence editor.

Table 1. List of specimens used in this study with locality and voucher information, ITS literature citation for previously published sequences, and GenBank accession numbers for both ITS and *trn*L-F.

Species	Voucher	ITS citation	Genbank ITS	Genbank *trn*L-F
Winteraceae				
Belliolum pancheri (Baill.) Vink (= *Zygogynum pancheri* (Baill.) Vink)	New Caledonia, Mt. Koghis, *LBT 205*, NO	Suh et al., 1993	AY004119	AY004135
Bubbia comptonii (Baker f.) Dandy (= *Zygogynum comptonii* (Baker f.) Vink)	New Caledonia, Prokoméo, NW of Canala, *LBT 204*, NO	Suh et al., 1993	AY004123	AY004140
Drimys winteri J.R. Forst. & G. Forst.	South America, Chile, (Berkeley B. G.) 45.307, NO	Suh et al., 1993	AY004126	AY004143
Exospermum stipitatum (Baill.) Tiegh. ex Morot (= *Zygogynum stipitatum* Baill.)	New Caledonia, Mt. Panié, *LBT 202*, NO	Suh et al., 1993	AY004121	AY004138
Pseudowintera axillaris (J.R. Forst. & G. Forst.) Dandy	New Zealand, N. Island, Akatarawa, *LBT 300*, NO	Suh et al., 1993	AY004124	AY004141
Pseudowintera colorata (Raoul) Dandy	New Zealand, N. Island, Akatarawa, *LBT 301*, NO	Suh et al., 1993	AY004125	AY004142
Takhtajania perrieri (Capuron) Baranova & J.-F. Leroy	Madagascar, Anjahanaribe-Sud Special Reserve, *Rakotomalaza et al. 1342*, MO	This paper	AY004129	AY004146
Tasmannia insipida R.Br. ex DC.	Australia, Queensland, Atherton, *LBT 108*, NO	Suh et al., 1993	AY004127	AY004144
Tasmannia lanceolata (Poir.) A.C. Smith	Australia, (Berkeley B. G.) 60.0052, NO	Suh et al., 1993	AY004128	AY004145
Zygogynum acsmithii Vink	New Caledonia, Mts. near Lac Enhuit, *LBT 201*, NO	Suh et al., 1993	AY004122	AY004139
Zygogynum balansae Tiegh. (= *Zygogynum pomiferum* Baill. subsp. *balansae* (Tiegh.) Vink)	New Caledonia, Dzumac Mts., *LBT 203*, NO	Suh et al., 1993	AY004120	AY004137
Zygogynum bicolor Tiegh.	New Caledonia, Plateau de Dogny, *LBT 200*, NO	Suh et al., 1993	AY004118	AY004135
Canellaceae				
Canella winterana (L.) Gaertn.	South America, *LBT 124*, NO	Suh et al., 1992	L03844	AY004152
Capsicodendron dinisii (Schwacke) Occhioni	South America, *Butzke et al. 11521*, US	This paper	AY004132	AY004149
Cinnamodendron ekmanii Sleumer	Dominican Republic, Samaná Penninsula, *García & Veloz 6866*, SD	This paper	AY004133	AY004150
Cinnamosma madagascariensis Danguy	Madagascar, *Lowry 4991*, MO	This paper	AY004131	AY004148
Pleodendron macranthum (Baill.) Tiegh.	Puerto Rico, *Axelrod 10783*, MO	This paper	AY004134	AY004151
Warburgia salutaris (G. Bertol.) Chiov.	South Africa, Mpumalanga, *Goldblatt 11314*, MO	This paper	AY004130	AY004147

Nuclear ITS. The alignment of Winteraceae ITS sequences of Suh et al. (1993) was used as a guide to easily incorporate the *Takhtajania* ITS sequence. The paralogous copies (GenBank AY004111–AY004117) of the ITS region as determined by Buckler et al. (1997) were not included in the alignment.

Alignment of ITS sequences within Canellaceae also was easily determined by eye. Alignment of Canellaceae with Winteraceae sequences into a single data set required additional information to generate a plausible alignment. One region of ITS 1 and another in ITS 2 were unalignable across both families, though these regions were alignable

among families. These regions were treated as non-overlapping insertion/deletion (indel) events in the final data set. Inferred indel regions were included in all phylogenetic analyses with the resulting gaps treated as missing.

Putative secondary structures of ITS 2 were determined for each taxon employing the minimum free-energy program mFOLD (Zuker, 1989) using constraints previously described by Hershkovitz and Zimmer (1996). In their study, the putative secondary structure of *Canella* ITS 2 was determined, and this structure was used as a guide to determine structures for all remaining Canellaceae.

Chloroplast trn*L-F*. The *trn*L-F spacer regions were easily aligned by eye within and between both families, with the resulting indels treated identically to those in the ITS data set.

Combined data set. The aligned ITS and *trn*L-F spacer sequences were then combined into a single data set. Two partitions were defined (ITS and *trn*L-F) and subjected to the data partition-homogeneity test (Farris et al., 1995). A thousand replicates were performed and the resulting P-value was used to determine if using the combined data set for phylogenetic reconstruction would be appropriate.

In summary, three data sets were examined: (1) the ITS data set consisting of the ITS 1, 5.8S, and ITS 2 regions; (2) the *trn*L-F data set consisting of a group I intron, the 5′-*trn*L exon, a non-coding intergenic spacer region, and partial sequence of *trn*F; and (3) a combined data set consisting of both the ITS and *trn*L-F regions.

PHYLOGENETIC ANALYSES

All phylogenetic reconstruction analyses were conducted using version 4.0b1 or 4.0b2 of PAUP* (Swofford, 1998).

Maximum parsimony. For each data set, phylogenetic reconstruction under maximum parsimony (MP) was conducted by utilizing the Branch and Bound search option in PAUP* with TBR branch-swapping, MULPARS, and ACCTRAN options active. Characters were assigned equal weights at all nucleotide positions (Fitch, 1971). Robustness of cladistic linkages was evaluated with 1000 bootstrap replicates (Felsenstein, 1985; Sanderson, 1989). Decay values (Bremer, 1988; Donoghue et al., 1992) for each node were calculated using the TOPOLOGICAL CONSTRAINTS option in PAUP*.

Maximum likelihood. The strategy employed utilized the likelihood-ratio test statistic (Felsenstein, 1981; Goldman, 1993; Yang et al., 1995) to determine the best model of DNA substitution for each of the three data sets described above. The models considered include the general time-reversible model (GTR, equals REV of Yang, 1994a), Hasegawa et al. (1985; denoted HKY85), Kimura (1980; denoted K2P), and Jukes and Cantor (1969; denoted JC69). Equations for calculating likelihoods under these DNA substitution models are given in Swofford et al. (1996). An iterative procedure to first evaluate models and optimize model parameters was used on an initial set of trees generated from the maximum parsimony analysis.

For each model of DNA substitution, models that incorporate rate variation across sites were explored. These included equal rates assumed across all sites, a proportion of sites assumed to be invariable with equal rates assumed across variable sites ("I" [Hasegawa et al., 1985]), all sites assumed to follow a discrete approximation of the gamma distribution ("Γ" [Yang, 1994b]), and some sites assumed to be invariable with gamma distributed rates at variable sites ("I + Γ" [Gu et al., 1995; Waddell & Penny, 1996]).

For the combined data set, a set of models was tested which explored the possibility that the relative rates between the two molecules differed significantly. This class of models assigns sites to classes and then estimates the relative rate for each class separately (Felsenstein, 1991; Goldman & Yang, 1994). Sites were assigned to two classes (ITS and *trn*L-F). This model is denoted as "+ SS."

For each data set, a maximum likelihood heuristic search with TBR branch swapping was then performed using parameters estimated from the tree with the best likelihood score under the best model. If the resulting tree was different in topology from that of the original tree, the resulting tree was used to further optimize the model parameters. With parameters re-optimized, a heuristic search of ten repetitions of random taxon addition and TBR branch swapping was performed using fully defined model parameters. Bootstrap methods (Felsenstein, 1985; Sanderson, 1989) with 1000 replicates were performed to estimate robustness of nodal support.

RESULTS

The combined data set used in this study can be found at TreeBASE (http://www.herbaria.harvard.edu/treebase/).

SEQUENCE ALIGNMENT

Nuclear ITS. ITS sequence alignment proved to be a relatively simple task within both Winteraceae and Canellaceae. With the exception of two regions, one in ITS 1 (bp 1143–1163) and the other in ITS

Table 2. List of the insertion/deletion (indel) events for the *trn*L-F/ITS combined data set. Character name (A–X for *trn*L-F; 1–115 for ITS), position information (character number in the combined data set), indel length (bp), character type (I = parsimony-informative, U = uninformative, H = homoplastic when mapped on Figs. 1 and 2a), and the species where indels were found are noted. Bp = *Belliolum pancheri*, Bc = *Bubbia comptonii*, Dw = *Drimys winteri*, Es = *Exospermum stipitatum*, Pa = *Pseudowintera axillaris*, Pc = *Pseudowintera colorata*, Tp = *Takhtajania perrieri*, Ti = *Tasmannia insipida*, Tl = *Tasmannia lanceolata*, Za = *Zygogynum acsmithii*, Zba = *Zygogynum balansae*, Zbi = *Zygogynum bicolor*, Cw = *Canella winterana*, Cd = *Capsicodendron dinisii*, Ce = *Cinnamodendron ekmanii*, Cm = *Cinnamosma madagascariensis*, Pm = *Pleodendron macranthum*, Ws = *Warburgia salutaris*.

Character	Position in combined data set	Length (bp)	I/U/H	Clade
***trn*L-F**				
A	114	1	I	Winteraceae/Canellaceae
B	179–181	3	I	Winteraceae/Canellaceae
C	207–212	6	U	Tp
D	247–255	9	U	Cw
E	252–256	5	I	Winteraceae/Canellaceae
F	257–264	8	U	Ws
G	282–286	4	U	Cm
H	317	1	U	Cw
I	319–331	13	U	Cw
J	323–331	9	U	Tp
K (a, b, c)	359–364	1	H	a = (Ti, Tl, Tp), b = (Tl, Tp), c = Tl
L	379–384	6	I	Dw, Pc, Pa, Bc, Bp, Es, Za, Zba, Zbi
M	385–388	4	U	Cd
N	414–418	5	I	Winteraceae/Canellaceae
O	586–591	6	H	Run of Adenines (see text)
P	590–694	105	I	Winteraceae/Canellaceae
Q	633	1	U	Dw
R	634	1	U	Tp
S	706	1	I	Winteraceae/Canellaceae
T	710	1	I	Winteraceae/Canellaceae
U	717–718	2	I	Winteraceae/Canellaceae
V	740–750	11	U	Ws
W	772	1	U	Ti
X	897	1	H	Cd, Cm, Ws
ITS-1				
1	993	1	I	Winteraceae/Canellaceae
2	997	1	I	Bc, Bp, Es, Za, Zba, Zbi
3	998–999	2	I	Cd, Ce, Cm, Pm, Ws
4	1000	1	U	Cd
5	1003–1013	11	U	Cd
6	1002	1	I	Pa, Pc
7	1003	1	U	Tp
8	1008	1	I	Winteraceae/Canellaceae
9	1009	1	U	Cw
10	1027–1028	2	I	Winteraceae/Canellaceae
11	1040	1	U	Dw
12	1041	1	I	Winteraceae/Canellaceae
13	1042	1	H	Winteraceae, Cd
14	1045	1	I	Cd, Cm, Ws
15	1048–1049	2	H	Canellaceae, Dw
16	1050–1051	2	I	Winteraceae/Canellaceae
17	1052	1	I	Cd, Cm, Ws
18	1053–1054	2	U	Ws
19	1058	1	H	Canellaceae, Dw
20	1059	1	H	Cd, Dw

Table 2. Continued.

Character	Position in combined data set	Length (bp)	I/U/H	Clade
21	1060–1061	2	U	Dw
22	1068	1	U	Cw
23	1076–1077	2	U	Cw
24	1081–1082	2	I	Tl, Ti
25	1083–1084	2	I	Winteraceae/Canellaceae
26	1090	1	H	Winteraceae, Cm, Pm
27	1091	1	H	Ce, Cw, Pm
28	1094–1106	13	U	Cd
29	1194–1097	4	U	Ws
30	1198–1104	7	H	Ce, Cw, Pm, Ws
31	1105–1106	2	U	Pm
32	1112–1113	2	U	Dw
33	1114	1	I	Winteraceae, Cw
34	1115	1	I	Winteraceae/Canellaceae
35	1116–1118	3	I	Winteraceae, Cw
36	1119–1122	4	I	Winteraceae/Canellaceae
37	1128	1	U	Cw
38	1135	1	I	Winteraceae/Canellaceae
39	1141–1142	2	H	Winteraceae, Cd, Cw
40	1143–1146	4	I	Winteraceae/Canellaceae
41	1147–1163	17	I	Winteraceae/Canellaceae
42	1148–1149	2	U	Cd
43	1158	1	I	Cm, Ws
44	1176	1	U	Cw
45	1185–1188	4	I	Winteraceae/Canellaceae
46	1199	1	U	Dw
47	1206–1225	20	I	Winteraceae/Canellaceae
48	1209–1212	4	I	Ce, Pm
49	1223–1225	3	I	Ce, Pm
50	1234	1	I	Winteraceae/Canellaceae
51	1244	1	U	Cw
52	1245	1	I	Winteraceae/Canellaceae
53	1262–1277	16	I	Winteraceae/Canellaceae
54	1262–1263	2	I	Ce, Pm
55	1272	1	I	Cd, Cm, Ws
56	1273	1	I	Ce, Pm
57	1283–1284	2	H	Ce, Cw, Pm
58	1287	1	H	Cd, Cm, Cw, Ws
59	1292–1293	2	I	Bc, Bp, Es, Za, Zba, Zbi
60	1299	1	H	Cm, Pm
61	1308–1326	19	I	Winteraceae/Canellaceae
62	1311	1	I	Bp, Es, Za, Zba, Zbi
63	1312–1313	2	H	Bc, Pa, Ti, Tl, Tp
64	1314–1316	3	U	Tp
65	1325	1	H	Winteraceae, Ws
66	1326	1	H	Cd, Cm, Cw
67	1331	1	U	Cw
5.8S				
68	1487	1	U	Cw
ITS-2				
69	1498	1	H	Pa, Pc, Bc, Es, Za, Zba, Zbi
70	1504	1	I	Winteraceae/Canellaceae
71	1511–1513	3	I	Winteraceae/Canellaceae
72	1514	1	H	Cd, Ce, Cm, Pm
73	1523	1	H	Cd, Ce, Cw, Pm, Ws

Table 2. Continued.

Character	Position in combined data set	Length (bp)	I/U/H	Clade
74	1524–1525	2	H	Bc, Bp, Cm, Es, Ti, Tl, Za, Zba, Zbi
75	1526	1	I	Bc, Bp, Cm, Es, Za, Zba, Zbi
76	1527	1	I	Winteraceae/Canellaceae
77	1534	1	U	Dw
78	1535–1539	5	I	Winteraceae/Canellaceae
79	1540	1	H	Canellaceae, Bc
80	1543–1544	2	U	Cw
81	1545	1	I	Winteraceae/Canellaceae
82	1553–1554	2	I	Pa, Pc
83	1573–1575	3	I	Winteraceae/Canellaceae
84	1595–1596	2	I	Bp, Zb
85	1603	1	H	Bc, Bp, Ce, Cm, Es, Pm, Ti, Tl, Za, Zba, Zbi
86	1604	1	I	Bc, Bp, Cm, Es, Ti, Tl, Za, Zba, Zbi
87	1612–1618	7	U	Cw
88	1651	1	U	Tp
89	1652–1653	2	I	Winteraceae/Canellaceae
90	1659	1	I	Winteraceae/Canellaceae
91	1662	1	U	Pm
92	1663	1	I	Winteraceae/Canellaceae
93	1672	1	H	Winteraceae, Cm
94	1676	1	I	Cd, Cm, Ws
95	1683–1688	6	U	Ce
96	1690–1691	1	I	Pa, Pc
97	1692–1706	15	I	Winteraceae/Canellaceae
98	1692–1695	4	U	Tl
99	1700–1701	2	U	Tp
100	1707–1722	16	I	Winteraceae/Canellaceae
101	1709	1	U	Ce
102	1713–1716	4	U	Cw
103	1717–1718	2	H	Cw, Pm
104	1719–1720	2	U	Ce
105	1726–1727	2	U	Tl
106	1730	1	I	Winteraceae/Canellaceae
107	1737	1	U	Pc
108	1738	1	U	Pa
109	1736–1748	10	U	Pc
110	1750–1759	11	I	Winteraceae/Canellaceae
111	1757–1758	2	U	Tp
112	1763–1764	2	H	Cw, Cd, Pm
113	1765–1774	10	I	Cd, Cm, Ws
114	1775	1	H	Cd, cm
115	1776–1778	3	U	Cd

2 (bp 1690–1722), the alignment between families was also relatively straightforward. The two problematic regions were partitioned equally between the two families as non-overlapping separate indels (e.g., positions 1143–1153 were coded as gaps in Winteraceae and positions 1154–1163 were coded as gaps in Canellaceae). The effect of this was to reduce the probability of incorrect homology assessment between families while retaining potential phylogenetic information within each family.

Putative secondary structures for ITS 2 were determined for each sequence, and these structures were generally consistent with the final alignment (data not shown). An apparent contradiction between secondary structure and DNA alignment was found in the v5 region (see Hershkovitz & Zimmer

[1996] for details) and corresponds to bp 1704–1734. Although this region consistently formed a bulge in secondary structure analyses across all individuals, the base composition and sequence length clearly differed between families (11–15 bp with 46–60% G/C in Winteraceae and 7–16 bp with 75–85% G/C in Canellaceae). This result further supports treatment of this region as non-overlapping indel events. No known secondary structure exists for ITS 1; consequently, structural evidence supporting the alignment of ITS 1 could not be generated.

A total of 115 indels were identified in the ITS alignment, of which 53 (46%) were parsimony-informative, 38 (33%) were autapomorphic, and the remaining 24 (21%) were homoplastic. Table 2 presents these indels along with position information (character number in the data set), indel length, and the species where indels were found.

Chloroplast trn*L-F*. The *trn*L-F spacer regions were easily aligned by eye within and between families. Overall, a total of 24 indels (Table 2: A–X) were identified in the alignment. Of them, 9 (37.5%) were parsimony-informative, 12 (50.0%) were autapomorphic, and the remaining 3 (12.5%) were homoplastic. Especially noteworthy was indel P (Table 2) with 105 bases present in the Winteraceae, including *Takhtajania*, but this indel was not present in the Canellaceae.

Combined data set. Two partitions of the combined alignment were defined, (1) *trn*L-F (bp 1–987) and (2) ITS (bp 988–1778). These partitions were tested for congruence with the data partition-homogeneity test (1000 replicates) and yielded a P value of 0.367. This result fails to reject the null hypothesis of congruence between the two partitions. Therefore, all phylogenetic analyses were performed on both the individual and combined data sets.

PATTERNS OF SEQUENCE EVOLUTION

The aligned combined data set was 1778 characters long. Positions 1 to 987 represented the *trn*L-F chloroplast sequence region; positions 988 to 1778 represented the ITS nuclear ribosomal sequence region including the 5.8S coding region. Unlike the situation for certain genera of Winteraceae (*Belliolum, Bubbia, Exospermum,* and *Zygogynum*), no polymorphisms were detected in the ITS sequence for *Takhtajania* or any member of the Canellaceae.

The ITS 1 sequences in Winteraceae ranged from 238 bp in *Takhtajania* to 252 bp in *Drimys*. In Canellaceae, ITS 1 sequences ranged from 244 bp in *Capsicodendron* to 274 bp in *Pleodendron*. The 5.8S coding region was 164 bp in all individuals of both families, although it was only 163 bp long in *Canella*. ITS 2 sequences in Winteraceae ranged from 215 bp in *Pseudowintera colorata* to 228 bp in *Belliolum*, with *Takhtajania* having a 222 bp sequence. Canellaceae ITS 2 sequences ranged from 187 bp in *Cinnamodendron* to 211 bp in *Canella*.

The Winteraceae chloroplast group I intron sequences ranged from 490 bp in *Tasmannia insipida* to 508 bp in *Takhtajania perrieri*. In Canellaceae, these sequences ranged from 477 bp in *Canella winterana* to 495 bp in *Cinnamosma madagascariensis*. The 5′ *trn*L exon sequences were 50 bp in all individuals of both families. The intergenic spacer sequences in Winteraceae ranged from 360 bp in *Drimys* to 368 bp in both *Tasmannia insipida* and *Takhtajania perrieri*. All Canellaceae sequences were markedly shorter, ranging from 247 bp in *Warburgia salutaris* to 259 bp in *Canella winterana, Cinnamodendron ekmanii,* and *Pleodendron macranthum*. The 40 bp partial *trn*F exon sequences were identical in length in all individuals of both families.

PHYLOGENETIC ANALYSES

Maximum parsimony

Nuclear ITS. Using the Branch and Bound search procedure in PAUP*, with equal weighting of positions and Canellaceae selected as the outgroup, a single most parsimonious tree of 489 steps (366 steps excluding uninformative characters) was obtained with the ITS data set. The consistency index (Kluge & Farris, 1969) was 0.8221 (0.7623 excluding uninformative characters) and retention index (Farris, 1989) was 0.8793. Overall, 317 variable nucleotide positions were counted; of these, 206 (or 26% of the 791 total characters) provided parsimony-informative changes.

To assess robustness of these results, the bootstrap procedure (1000 replicates) and decay analyses were run in PAUP* (TOPOLOGICAL CONSTRAINTS were used to determine decay values for each node). These results are shown in Figure 1.

A polytomy including *Belliolum, Exospermum,* and *Zygogynum* (*Zygogynum* s.l.) was supported as monophyletic and sister to *Bubbia* by a 97% bootstrap value and decay index of + 4. The *Bubbia* + *Zygogynum* s.l. clade was monophyletic and sister to *Pseudowintera* (represented by two species, monophyletic in 99% of the bootstrap iterations [decay = + 5]), supported by a 100% bootstrap value (decay = + 10). *Drimys* was sister to this

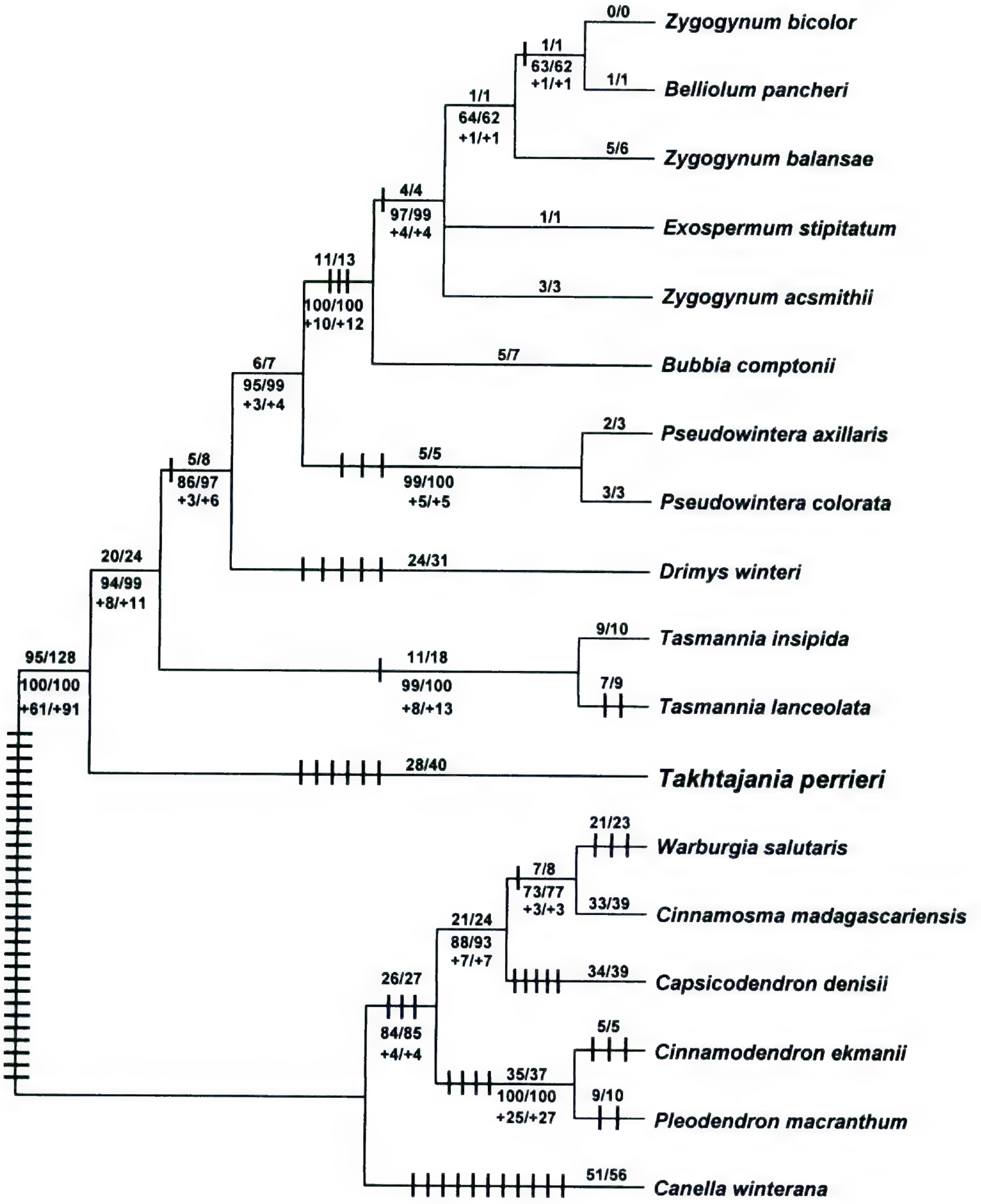

Figure 1. Single most parsimonious tree generated both from the ITS data set alone and the combined ITS/*trn*L-F data set. Tree length for ITS tree = 489 steps (366 steps excluding uninformative characters), consistency index = 0.8221 (0.7623 excluding uninformative characters), and retention index = 0.8793. Length of combined tree = 593 steps (434 steps excluding uninformative characters), consistency index = 0.8381 (0.7788 excluding uninformative characters), and retention index = 0.8994. Numbers to the left of the slash represent those generated from ITS data alone, while numbers to the right of the slash represent those generated from the combined data set. Branch lengths shown above branches, bootstrap values (1000 replicates) and decay values shown below branches, respectively. Dark bars represent non-homoplastic insertion/deletion events inferred from the ITS alignment (see Table 2).

assemblage in 95% of the bootstrap iterations (decay = + 3). *Tasmannia* (represented by two species) was monophyletic with 99% bootstrap support (decay = + 8) and sister to the *Drimys* + *Pseudowintera* + *Bubbia* + *Zygogynum* s.l. clade in 86% of the bootstrap iterations (decay = + 3). *Takhtajania* was found sister to the remainder of the Winteraceae with a bootstrap value of 94% (decay = + 8).

In the Canellaceae, *Cinnamosma* and *Warburgia* formed a clade sister to *Capsicodendron* in 73% of the bootstrap replicates, with a decay index of + 3. *Cinnamodendron* and *Pleodendron* formed a well-supported clade (100% of the bootstrap replicates, decay = + 25) sister to the *Capsicodendron* + *Cinnamosma* + *Warburgia* clade in 88% of the replicates (decay = + 7). Finally, *Canella* was found sister to the remainder of the family with 84% bootstrap support (decay = + 4).

Chloroplast trn*L-F*. Using the Branch and Bound search procedure in PAUP*, with equal weighting of positions and Canellaceae selected as the outgroup, two most parsimonious trees of 103 steps (67 steps excluding uninformative characters) were obtained with the *trn*L-F data set. The consistency index was 0.9223 (0.8806 excluding uninformative characters), and the retention index was 0.9657. Overall, 90 variable nucleotide positions were counted; of these, 55 (or 5.6% of the 987 total characters) provided parsimony-informative changes. These two trees are presented in Figure 2 along with bootstrap values and decay indices.

With the exception of alternative equally parsimonious basal relationships, the topology generated by the *trn*L-F sequence data for Winteraceae was not in conflict with the tree generated by the ITS data, albeit there were both lower resolution and lower bootstrap support values. The polytomy of *Belliolum*, *Exospermum*, and *Zygogynum* (*Zygogynum* s.l.) expanded to include *Bubbia* (78% bootstrap and decay = + 2). The two species of *Pseudowintera* were unresolved with respect to each other and to the *Bubbia* + *Zygogynum* s.l. polytomy, and together these were only weakly supported as monophyletic and sister to *Drimys* (69% bootstrap and decay = + 1). The two species of *Tasmannia* were strongly supported as sister taxa in 98% of the bootstrap iterations (decay = + 5). Finally, the *trn*L-F sequence data could not resolve basal relationships within the family; both *Takhtajania* (Fig. 2a) and *Tasmannia* (Fig. 2b) as sister to the remaining Winteraceae were equally parsimonious.

Within Canellaceae, except for a strong sister relationship between *Cinnamodendron* and *Pleodendron* (88% bootstrap and decay = + 2), the topology generated by *trn*L-F data resulted in much lower levels of bootstrap support, and, moreover, suggested a fundamental split between Old World and New World lineages. Thus, rather than being sister to the remaining Canellaceae as suggested by the ITS-based phylogeny, *Canella* was nested within a weakly supported clade of New World genera. To force such a division of the family into Old World and New World clades requires an additional 11 steps in the ITS-based phylogeny.

Combined data set. Using the Branch and Bound search procedure in PAUP*, with equal weighting of positions and all Canellaceae genera selected as outgroups, a single most parsimonious tree of 593 steps (434 steps excluding uninformative characters) was obtained with the ITS/*trn*L-F combined data set. The consistency index was 0.8381 (0.7788 excluding uninformative characters) and the retention index was 0.8994. The topology derived from this combined data set was identical to that generated from the ITS data alone. The branch lengths, bootstrap values, and decay indices are presented in Figure 1.

Overall, with the addition of the *trn*L-F data, bootstrap values either remained similar or increased in comparison with the ITS-based phylogeny. Within the Winteraceae, the most notable increases resulted in stronger support for basal relationships in the family. The position of *Takhtajania* as basal and sister to the rest of the family was further strengthened (ITS = 94% bootstrap and + 8 decay index vs. combined data = 99% bootstrap and + 11 decay index), as was the position of *Tasmannia* as the next branch within the family. The combined data set provided especially strong support for the inclusion of *Drimys* in the clade consisting of all other members of the family and sister to *Tasmannia* (ITS = 86% bootstrap and + 3 decay index vs. combined data = 97% bootstrap and + 6 decay index). Within the Canellaceae, bootstrap support also remained similar or increased only slightly with the addition of the *trn*L-F data (Fig. 1).

Maximum likelihood

Nuclear ITS. For the ITS data set, the best model of DNA substitution was the general time-reversible model with a proportion of sites assumed to be invariable and equal rates assumed across variable sites (GTR + I). With this model, parameters were fully optimized using the tree generated from the parsimony search (Fig. 1). With the model parameters defined, a heuristic search was con-

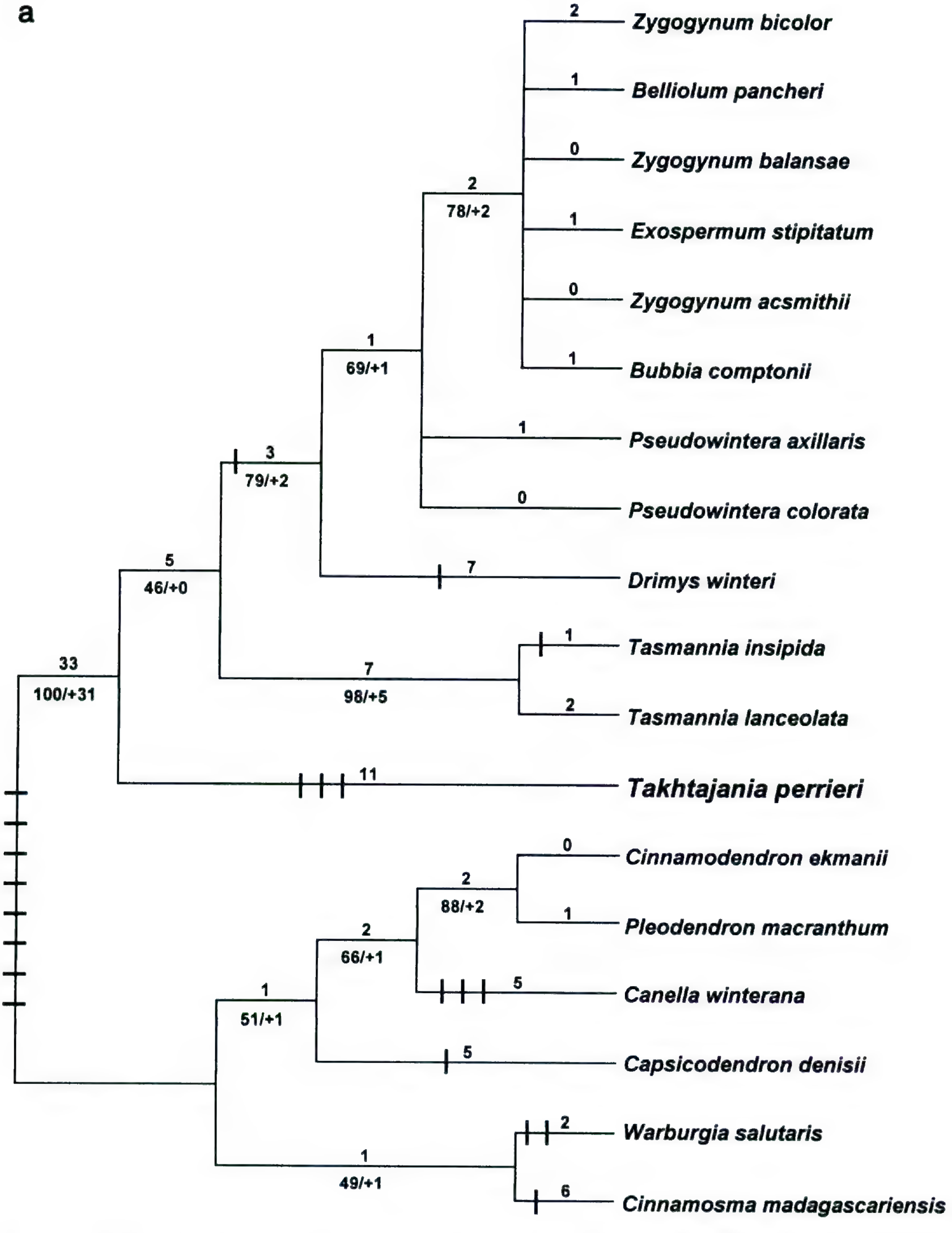

Figure 2a, b. Two most parsimonious trees generated using *trn*L-F data. Tree length = 103 steps (67 steps excluding uninformative characters), consistency index = 0.9223 (0.8806 excluding uninformative characters), and retention index = 0.9657. Branch lengths shown above branches, bootstrap values (1000 replicates) and decay values shown below branches, respectively. Dark bars (Fig. 2a) represent non-homoplastic insertion/deletion events inferred from the *trn*L-F alignment (see Table 2).

b

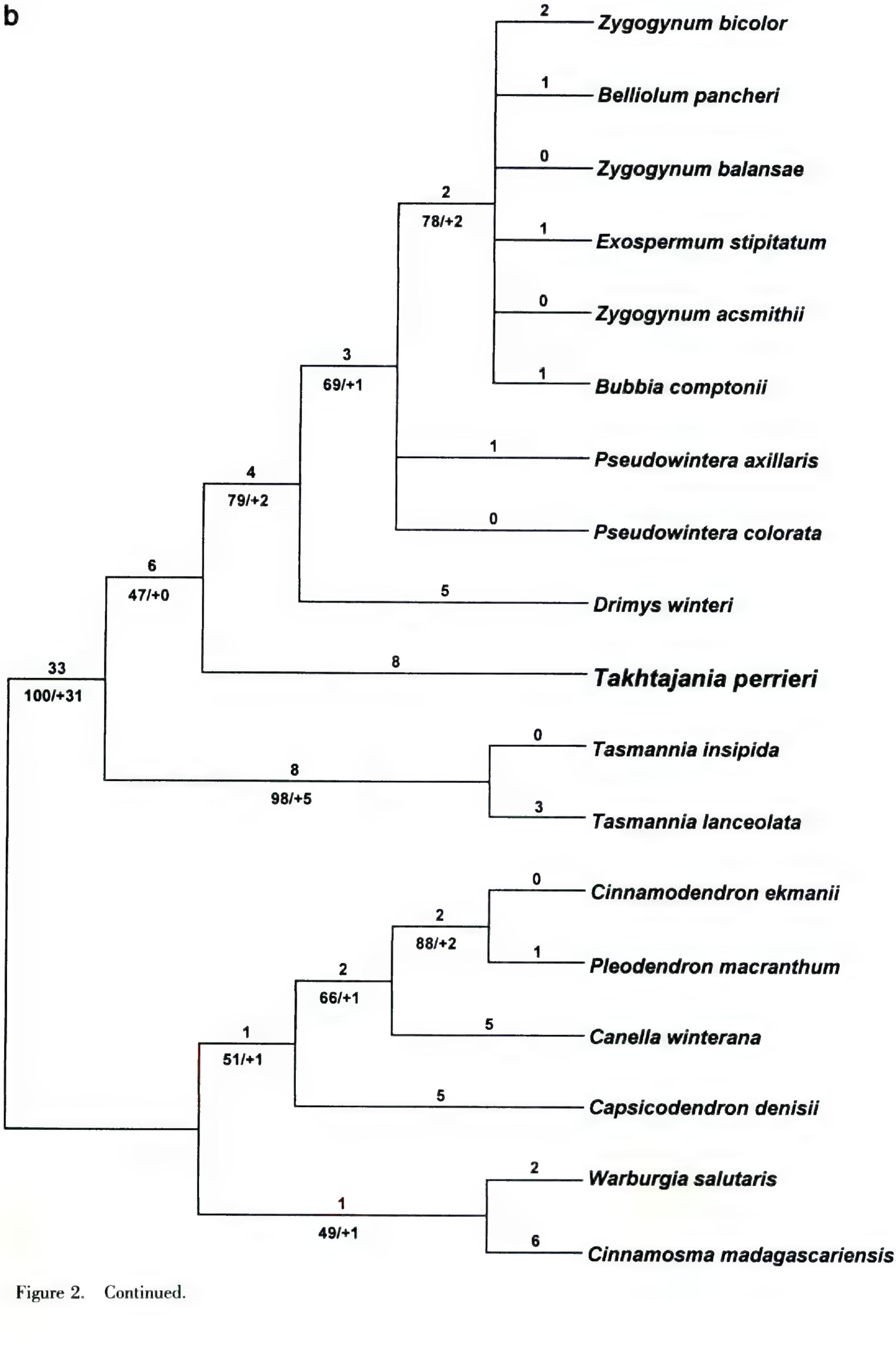

Figure 2. Continued.

a

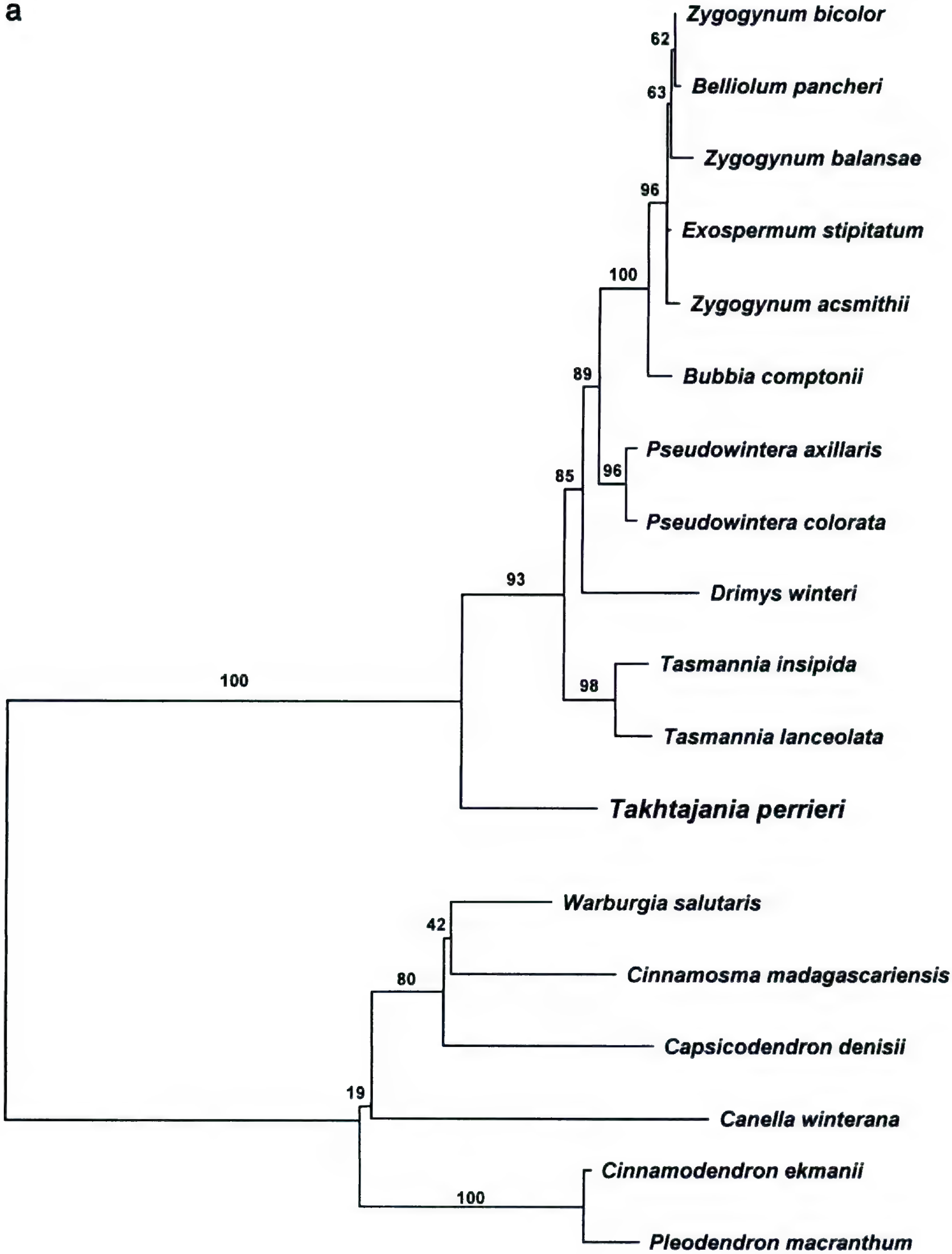

Figure 3a. One of four most likely phylograms generated with ITS data (−ln = 3371.16) with bootstrap values (1000 replicates) above branches.

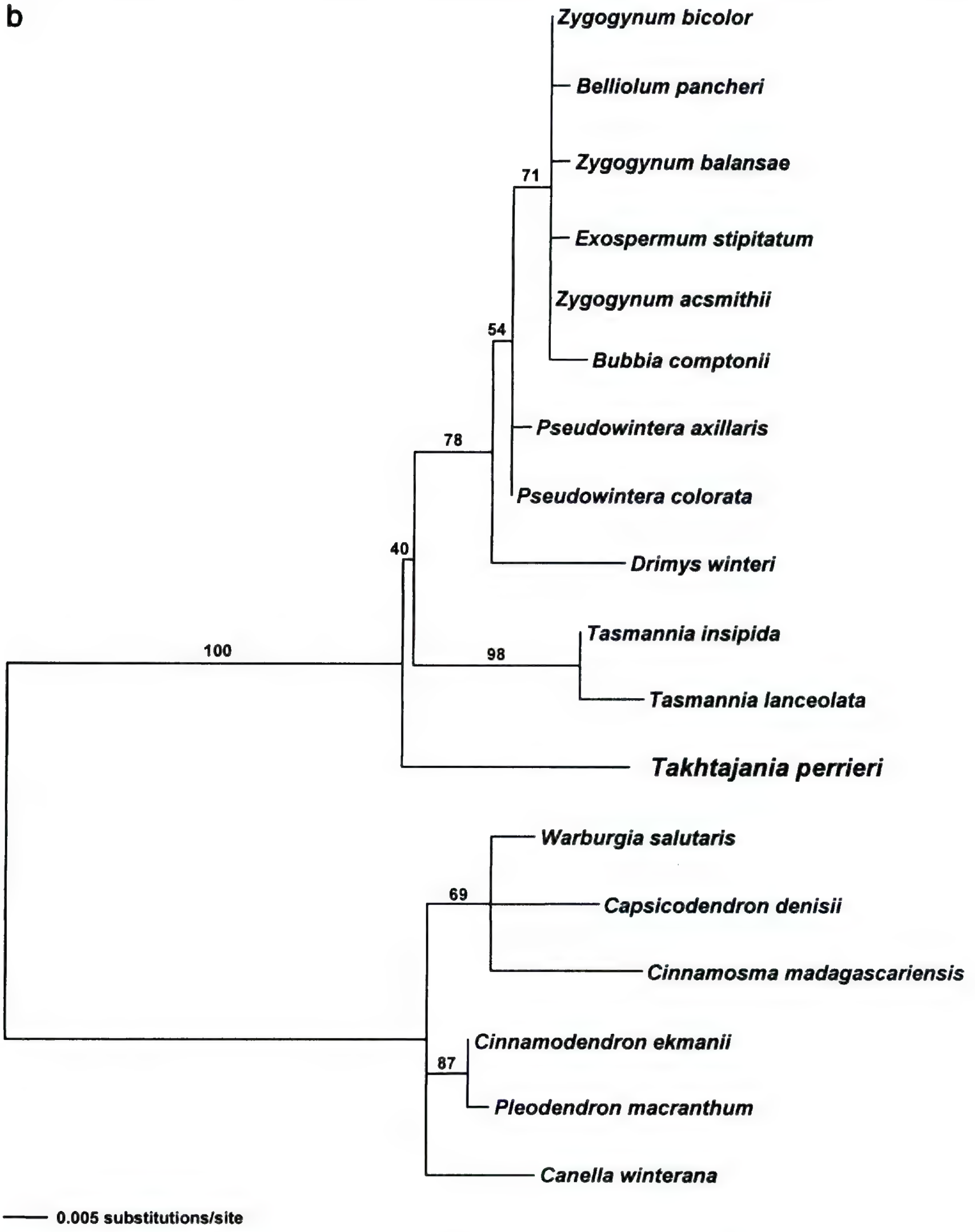

Figure 3b. Single most likely phylogram generated with *trn*L-F data (−ln = 1996.3473) with bootstrap values (1000 replicates) above branches. Figure 3c is on page 428.

ducted that resulted in three trees, one of which was identical in topology to the most parsimonious tree (Fig. 1). These three trees were used to further optimize model parameters. The resulting parameter values did not differ from those generated by using the single maximum parsimony tree. Thus, ten heuristic searches (with random taxon addition, multrees, and steepest descent active) were conducted using the parameters defined in the initial search. These ten random taxon addition searches generated a total of four equally likely trees (−ln = 3371.16), one of which is presented in Figure 3a.

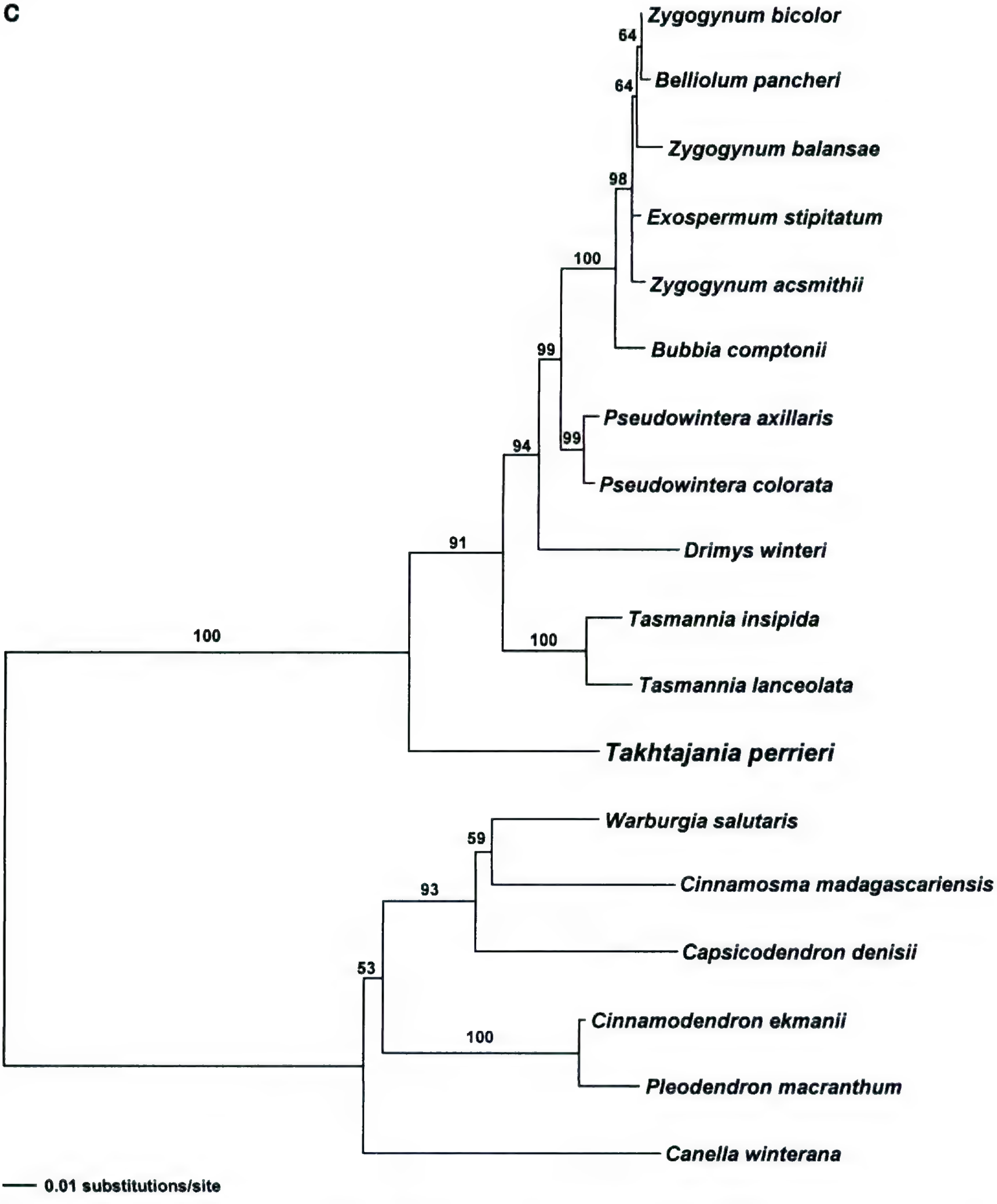

Figure 3c. Single most likely phylogram generated with combined ITS/*trn*L-F data set (ln = −5508.26165) with bootstrap values (1000 replicates) above branches.

In all four trees the relationships within Winteraceae were identical to those generated by the MP search, with *Takhtajania* sister to the remainder of the Winteraceae. The four trees differed in the position of *Canella* in the Canellaceae. Either *Canella* was sister to the *Warburgia* + *Cinnamosma* + *Capsicodendron* clade, or sister to the *Cinnamodendron* + *Pleodendron* clade, or sister to the remainder of the family, or, finally, in an unresolved polytomy with the three above-mentioned clades. Although *Canella* is included within a monophyletic Canellaceae in 100% of the bootstrap iterations, bootstrap values do not support a resolved phylogenetic position of *Canella* within the family.

Chloroplast trn*L-F*. For the *trn*L-F data set, the best model of DNA substitution was the HKY85

model with a proportion of sites assumed to be invariable and equal rates assumed across variable sites (HKY85 + I). With this model, parameters were fully optimized using the two trees generated from the parsimony search (Fig. 2a, b). With the model parameters defined, a heuristic search was conducted that resulted in a single tree identical in topology to one of the four most likely trees generated from the maximum likelihood ITS search (Fig. 3a). Using this tree, parameters were re-estimated and the resulting optimized values were used for ten heuristic searches (random taxon addition, multrees, and steepest descent active). The result of these searches generated a single most likely tree ($-\ln = 1996.3473$), which is presented in Figure 3b along with bootstrap values for each node.

The single tree from the maximum likelihood search was identical to one of the two trees generated by the maximum parsimony analysis using *trn*L-F with one exception. The placement of *Canella* sister to *Pleodendron* and *Cinnamodendron* was not supported. Rather, *Capsicodendron, Cinnamosma,* and *Warburgia* were supported as an unresolved trichotomy in 69% of the bootstrap iterations. *Cinnamodendron* and *Pleodendron* were supported as sister taxa in 87% of the bootstrap iterations, and finally *Canella* was found in an unresolved trichotomy with the two above-mentioned clades in a monophyletic Canellaceae in 100% of the bootstrap replications. Within the Winteraceae an unresolved polytomy including *Belliolum, Bubbia, Exospermum,* and *Zygogynum* was found to be sister to the two species of *Pseudowintera* in 71% of the bootstrap replicates. In 78% of the bootstrap iterations, *Drimys* joined the above in a monophyletic clade, but was only weakly supported by a 54% bootstrap value as sister to them. There was less than 50% bootstrap support for the basal position of *Takhtajania* sister to the remainder of Winteraceae.

Combined data set. For the combined data set, the best model of DNA substitution was the general time-reversible model. Among site rate variation was accommodated for by dividing the data into two partitions (ITS and *trn*L-F), thus allowing among site rate variation to be estimated for each molecule separately (GTR + SS). With this model, parameters were optimized using the tree generated from the maximum parsimony search (Fig. 1). With the model parameters defined, a heuristic search was conducted that resulted in a single most likely tree identical in topology to the most parsimonious tree using the same data set (Fig. 1). This tree was used to further optimize model parameters. The resulting parameter values did not differ, and thus ten heuristic searches (random taxon addition, multrees, and steepest descent active) were conducted using the parameters defined in the initial search. These ten random taxon addition searches generated a single most likely tree ($\ln = -5508.26165$). The bootstrap values are presented in Figure 3c.

In general, with the exception of support for *Canella* as basal within Canellaceae, the bootstrap values were consistent with those generated by the parsimony analyses of the combined data set. In the parsimony analysis, *Canella* was sister to the remainder of the Canellaceae in 85% of bootstrap iterations, whereas the likelihood analyses recovered this relationship in only 53% of the bootstrap replicates.

Discussion

In the initial molecular phylogenetic study of Winteraceae, Suh et al. (1993) found it difficult to align the ITS region with certainty for several exemplar outgroup species. The present study increased generic sampling in Canellaceae from a single exemplar (*Canella*) to a representative of all genera, which greatly facilitated homology assessment between the two families. In addition, the more slowly evolving *trn*L-F chloroplast regions, for which no difficulty in alignment was encountered, were investigated. With Canellaceae as the outgroup, the rooting of Winteraceae is no longer ambiguous. Phylogenetic analyses of ITS and combined ITS/*trn*L-F DNA sequences provided a well-resolved molecular phylogeny for the Winteraceae that placed *Takhtajania* sister to the remaining genera in the family. Analysis of *trn*L-F sequence data alone failed to resolve the basal topology within the family, with either *Takhtajania* or *Tasmannia* equally parsimonious as the basal branch. Nevertheless, the addition of *trn*L-F sequences to a combined ITS/*trn*L-F analysis resulted in increased support values for the same topology derived from ITS sequences alone. Maximum likelihood analyses of each of the three data sets mirrored those obtained through maximum parsimony.

ITS and *trn*L-F sequences provided generally weaker support for phylogenetic relationships within Canellaceae. Although parsimony analysis of ITS data revealed moderate support for a basal position of *Canella* sister to the remaining members of the family, parsimony analysis of *trn*L-F data weakly suggested a fundamental New World/Old World split in Canellaceae. Parsimony analysis of the combined molecular data set resulted in a topology identical to that generated by ITS data alone, with only slight increases in bootstrap support and iden-

tical decay values. Maximum likelihood analyses of each of the three data sets further demonstrated the poor resolution these molecules provide for estimating basal relationships in Canellaceae. Nevertheless, ITS, combined ITS/*trn*L-F, and all maximum likelihood analyses strongly suggest that the southeastern Brazilian endemic *Capsicodendron* forms a clade with the African/Malagasy genera *Warburgia* and *Cinnamosma*.

The alignment of ITS and *trn*L-F sequences for the 12 species of Winteraceae and 6 species of Canellaceae revealed numerous probable insertion-deletion events. When mapped on the trees, these indels furnished additional support for certain clades. A total of 37 indels (29 in ITS [Fig. 1] and 8 in *trn*L-F [Fig. 2a]) support the separation of Winteraceae from Canellaceae. Three ITS indels support the monophyly of *Pseudowintera*, and three different ITS indels support the monophyly of the *Zygogynum* s.l. (*Bubbia*, *Belliolum*, *Exospermum*, and *Zygogynum*) assemblage. One indel each in both nuclear and chloroplast sequences (Figs. 1 and 2a, respectively) support inclusion of *Drimys* in the *Zygogynum* s.l. + *Pseudowintera* clade, and thus provide additional evidence of its distinctness from *Tasmannia*. Within Canellaceae, four ITS indels support the sister relationship between *Cinnamodendron* and *Pleodendron*, whereas a single ITS indel supports a sister relationship between African *Warburgia* and Malagasy *Cinnamosma*. The basal position of *Canella* in the family is further suggested by three ITS indels that support the monophyly of the remaining genera of Canellaceae. None of the indels found in the *trn*L-F Canellaceae sequences were phylogenetically informative. In total, 139 indels were scored overall and 114 (82%) were consistent with the trees generated from the sequence data. The remaining 25 were homoplastic and in some cases difficult to score. For example, in *trn*L-F sequences, long adenine runs occurred at varying numbers in closely related taxa. The length of these runs was not consistent when mapped on the tree, and delimiting homology was difficult when scoring this region. No indels were detected in either ITS or *trn*L-F that bear on the position of *Takhtajania* as sister to the remainder of Winteraceae.

Zygogynum s.l., *Pseudowintera*, and *Takhtajania* exhibit a short, early-rupturing involucrum (the congenitally fused two, or rarely three, outermost perianth parts, which could as well be considered the "calyx") that is persistent in fruit vs. the condition in both *Drimys* and *Tasmannia* where the ultimately caducous, late-rupturing involucrum becomes as long as the fully developed next inner tepal whorl, and therefore completely encloses the flower just prior to anthesis (Doust, 2000 this issue). A persistent, short calyx occurs in Canellaceae that is similar to the ruptured, persistent involucrum of basal *Takhtajania*, suggesting that *Pseudowintera* and *Zygogynum* s.l. have retained the ancestral plesiomorphic condition of an early-rupturing involucrum. In conjunction with the hypothesized phylogenetic positions of *Drimys* and *Tasmannia*, the differing ontogenetic patterns of tepal initiation and development exhibited by these two genera (Doust, 2000) imply that the late-rupturing, caducous involucrum has evolved twice independently. Similarly, deep red tepal color, present in *Canella* and other Canellaceae, as well as *Takhtajania* and many *Zygogynum* s.l. species, may be the ancestral condition, with the evolution of white tepals through the loss of pigmentation and/or the acquisition of an optical tapetum (Endress et al., 2000 this issue) occurring a number of times independently.

The historical biogeographical implications of *Takhtajania* in a basal position sister to the remaining genera of the Winteraceae have been discussed in the context of the distribution of fossil Winteraceae (Doyle, 2000 this issue) and ecophysiological constraints (Feild et al., 2000 this issue). It is reasonable to assume that the basal branch leading to *Takhtajania* became isolated in Madagascar after reaching there from continental Africa, and also that Madagascar may have played a prominent role in the migration of the family to Australasia and South America via Antarctica during the mid-Cretaceous. The presence of two different winteraceous pollen types in the Miocene of the southwestern Cape possibly referable to *Tasmannia* and the most advanced *Zygogynum* s.l. clade is more puzzling and problematic (Coetzee & Praglowski, 1988). With South Africa relatively isolated from both South America and Antarctica from the mid-Cretaceous onward (Smith et al., 1994), either all of the currently extant clades of Winteraceae had already evolved prior to that point in time, or Madagascar may have continued to serve as a conduit for migration of more advanced Winteraceae back into South Africa from Australasia. In comparison to the Southern Gondwanan pattern of radiation in Winteraceae, the preliminary phylogenetic hypothesis of infrafamilial relationships within Canellaceae suggests an "inverted" Northern Gondwanan biogeographical history. Basal clades of Canellaceae are centered in northern South America and the West Indies, with a more advanced clade exhibiting a southeastern Brazil/African–Malagasy split related to the middle Cretaceous sepa-

ration of South America and Africa (Goldblatt, 1993) and a subsequent dispersal event to Madagascar. Thus, the modern-day sympatric occurrence of the two Malagasy endemic genera *Cinnamosma* (Canellaceae) and *Takhtajania* (Winteraceae) may well be the end result of vastly divergent biogeographic histories.

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BOOK REVIEW

Ribeiro, J. S. da S. et al. 1999. Flora da Reserva Ducke. Guia de indentificação das plantas vasculares de uma floresta de terra-firme na Amazônia Central. 800 pp. Softcover. ISBN 85-211-0011-76. INPA (Brazil) and DFID (U.K.). Retail price: $50 U.S. (through http://www.balogh.com).

I am not normally one to gush praises on a new flora book, but this one deserves a score of 12 on a scale of 0 to 10 (I admit to giving extra credit on my exams). Why such a high rating? Because this book excels in a number of important criteria, such as high information content, richness of graphics, full-color pages, high degree of innovation, reasonable price, and all this for a small reserve near Manaus in central Amazonian Brazil that shares a good number of its species with the much broader Amazon basin and neighboring countries. The only downfall for some will be the Portuguese text, but the book is so graphics-oriented and self-guiding that it is still very useable by readers not familiar with that language. Still, an English translation of this book would be welcomed by many.

The book is a field guide—in the true sense—something you can actually bring into the field with you and use to identify forest plants. In fact, it conveniently comes with a transparent, thick plastic jacket that wraps around the open face of the book and secures to the rear dust cover with a Velcro strip. Neat! It is designed for ease of use, that is, non-technical and focused mainly on vegetative characters, since so often the plants (especially the trees) one encounters in tropical forests lack flowers or fruits at the time of one's visit. The flora covers 2175 species of vascular plants, each one illustrated by color photographs that are diagnostic of that particular group. For instance, nearly all trees have small photographs of the outer bark, a bark slash (to show exudates or inner cortex characters), an entire leaf, a detail of leaf venation, and often some other distinguishing feature such as a leaf gland or pulvinus. The species entries themselves do not include images of flowers or fruits, but at the beginning of each family, there are composite plates showing a large part of the diversity of flowers and fruits in the family members of the reserve (take a look at pages 152 to 155 to be amazed by the close-up photos of Lauraceae flowers and fruits!).

The Ducke Reserve is a 10 × 10 km forest preserve situated on the outskirts of Manaus. It has been the site of many research projects and silvicultural experiments over the past decades, but has now become a virtual island of forest amid the suburban sprawl of the capital of Brazilian Amazonia. The much broader geographical utility of the flora derives from the habitat diversity that is present in the reserve. There are four main vegetation types, associated with different soils and drainage patterns: (1) hilltop plateau forest, with clayey soils and good drainage; (2) slope forest, actually a gradient from the hilltop forest down to more sandy and poorly drained soils lower down; (3) "campinarana," or what might also be called "Amazonian caatinga," lower forests on white-sand spodosol soils; and (4) "baixio," or alluvial plains along streams that have poor drainage and are often waterlogged. Only small streams traverse the reserve, so it lacks the seasonally inundated areas known locally as "varzea" or "igapó."

I particularly recommend the illustrated glossaries, which cover pages 24 to 95. They are the best I have seen anywhere and include collages of small photographs of particular characters, for instance leaf glands, exudates, stilt and tabular root variations, leaf types and shapes, among many others. Identification works by a technique of nested diagram boxes with the characters written in or sometimes illustrated, leading to the actual species pages with further diagram boxes that lead to groups of species that can be perused and compared (there are short diagnostic text descriptions to accompany the graphics). Another endearing aspect of the book is feature boxes, or vignettes, interspersed in particular families. For instance, under Clusiaceae, a feature box (p. 253) illustrates and narrates the recently published story of parakeet pollination in *Moronobea coccinea*. There is also a valiant attempt at a "rapid key" that uses little colored markings on the right-hand margin of species pages to narrow down a series of criteria listed on pages 94 and 95. It is a great concept, though somewhat awkward in practice.

Let me cite a personal example of how useful this book is as a field guide. During a recent trip to San Carlos de Rio Negro, Venezuela, some 1000 km to the north, Gerardo Aymard and I collected a sterile canopy tree with bipinnate leaves, clearly a legume and to our eyes likely an *Abarema* species, but one we had never seen with such huge, "dixie-cup"-like petiolar glands. Returning to Caracas, I searched the manuscript of the Mimosaceae for the

Flora of the Venezuelan Guayana and then looked through the entire family at the National Herbarium (VEN), to no avail. It took me a mere five minutes to follow the keys through the Ducke Reserve book and come across a very likely candidate, *Abarema adenophora*. Upon consulting Barneby and Grimes's (1996) superb monograph of the genus, I confirmed the identification, the first report for Venezuela.

My only real quibble with the book is in the introductory section, where the authors briefly discuss the diversity and phytogeography of the reserve. As one of the causes explaining the high diversity of the area, they discuss and actually depict a hypothetical Pleistocene "Lago Amazonas" reaching from the base of the Andes to nearly the mouth of the Amazon and draining through the present-day Orinoco River to the Caribbean rather than into the Atlantic. The original paper postulating this lake (Frailey et al., 1988) presented some evidence of the existence of a lake in the *upper* Amazon (in Acre State, close to the border of Peru and Bolivia), but their extrapolation of the extent of the lake to the lower Amazon and draining out through the Orinoco was and continues to be highly speculative. However, a recent paper by Oliveira and Daly (1999), whose interpretation the Ducke book repeats, takes this shaky concept and makes it appear as widely accepted dogma. Likewise, the same section of the book steps into the "refugia" quagmire, asserting that the area of Manaus is a species refugium characterized by a high degree of local endemism with elements from many different phytogeographical regions. It is well known (Nelson et al., 1990) that Manaus is the epicenter of plant collecting efforts in the Amazon, and that other parts of the basin are so woefully undercollected or in fact totally *un*sampled that any such conclusions stand on very slippery ground.

These small details aside, this book is a truly masterful work, many years in the making, and deserving of all the praise and wide distribution it can get. Mike Hopkins, one of the 14 authors listed on the title page, merits special credit for managing the project and overseeing the team of very capable Brazilian botanists who carried out most of the work, both in the field and in the office. —Paul E. Berry, *Department of Botany, University of Wisconsin–Madison, 132 Birge Hall, 430 Lincoln Drive, Madison, Wisconsin 53706, U.S.A.*

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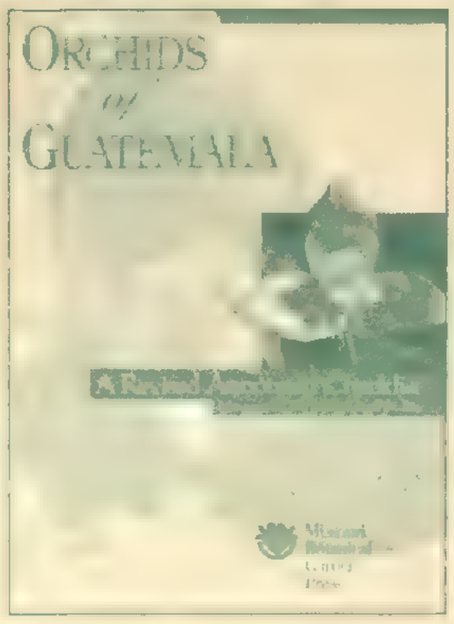

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PHYLOGENETIC RELATIONSHIPS WITHIN THE TRIBE JUSTICIEAE (ACANTHACEAE): EVIDENCE FROM MOLECULAR SEQUENCES, MORPHOLOGY, AND CYTOLOGY[1]

Lucinda A. McDade,[2] *Thomas F. Daniel,*[3] *Susan E. Masta,*[2] *and Katherine M. Riley*[2]

Abstract

We used molecular sequence data from the nuclear ribosomal internal transcribed spacers and from the intron and spacer of the *trn*L-*trn*F chloroplast region to study phylogenetic relationships within the large (ca. 2000 species), wide-ranging, and taxonomically difficult tribe Justicieae (Acanthaceae). The partition homogeneity test indicated that the data sets for the two loci were congruent, and separate analyses of the two gave similar results. Analysis of the combined data set provides a highly resolved hypothesis of relationships, much of it strongly supported. Justicieae are strongly supported as monophyletic; within the tribe, five lineages and one paraphyletic grade are related as follows: [*Pseuderanthemum* lineage (Isoglossinae {*Tetramerium* lineage [multiple clades of Old World "justicioids" (Diclipterinae + New World "justicioids")]})]. Many aspects of this phylogenetic hypothesis are supported by data from morphology and cytology, and some conform to earlier classifications of the group. There are, however, a number of novel aspects. Notably, the large genus *Justicia* (ca. 700 species) is not monophyletic; the Old World members form a grade and the New World members are monophyletic only if a number of other genera are included. The very strongly supported sister-group relationship between Diclipterinae and the New World "justicioid" lineage is novel, and we cannot identify non-molecular synapomorphies to confirm this relationship. *Rhinacanthus*, a "justicioid" (*Justicia* and morphologically similar genera) by all but phylogenetic criteria, is strongly supported as a basal member of Diclipterinae, and cytological evidence confirms this placement. The *Pseuderanthemum* lineage is only modestly supported as monophyletic and may, in fact, represent a series of basal lineages. These plants are marked by having four staminal elements (four stamens or two stamens plus two staminodes), a plesiomorphic condition for all Acanthaceae. Additional evidence (both taxa and characters) will be necessary to resolve this uncertainty, as well as to determine the phylogenetic status of Old World "justicioids." Our analysis does provide considerable resolution of relationships within monophyletic lineages, and these relationships are discussed in the context of non-molecular evidence and previous classifications.

Key words: Acanthaceae, cp *trn*L-*trn*F, cytology, Justicieae, Lamiales, molecular sequences, morphology, nr ITS, phylogenetics.

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"I will not say that . . . [my] classification of the Justicieae . . . is perfect. On the contrary, I am convinced that it is no more than a tentative effort" (Bremekamp, 1965: 30).

The tribe Justicieae is the largest and arguably most systematically challenging lineage in the plant family Acanthaceae. Justicieae comprise about 2000 species, and their nearly worldwide distribution essentially matches that of the family as a whole. As documented by Lindau (in 1895) and supplemented by numerous other contributions since that time, the macromorphological (especially habit and floral) and palynological diversity within the tribe is considerable. Composition, infratribal relationships, and affinities of Justicieae have been controversial ever since Lindau's (1895) comprehensive infrafamilial classification of Acanthaceae. Lindau grouped *Justicia* L. and several of its presumed close relatives into a tribe characterized by having an androecium of two stamens and "Knötchenpollen" (2- or 3-aperturate pollen with 1 to 3 rows of insulae on each side of the apertures). The fact that several taxa of Lindau's Justicieae do not have this type of pollen whereas several genera with two stamens and "Knötchenpollen" were classified in other tribes by Lindau is indicative of problems with his classification.

Tribe Justicieae was classified by Lindau as part of "Imbricatae," a supratribal group that was defined by imbricate corolla aestivation. This pattern of aestivation is likely primitive for Acanthaceae, and Imbricatae are a heterogeneous group, as has been recognized for some time (Bremekamp, 1965) and confirmed by recent phylogenetic work (Hedrén et al., 1995; Scotland et al., 1995; McDade & Moody, 1999; McDade et al., 2000). Further, Scotland et al. (1994) showed that there is more than one variant of imbricate aestivation among Acanthaceae. Other authors have demonstrated that aestivation varies within lineages (Manktelow et al., in press) and even within genera (Schönenberger & Endress, 1998) such that its use as a defining character may be unwarranted. Moreover, Lindau's (1895) tribes and subtribes of Imbricatae, including Justicieae as described above, are not clearly circumscribed morphologically, and many are disparate assemblages. The character basis for the classification is inconsistent in that characters used by Lindau in his keys sometimes conflict with those employed in descriptions of tribes or genera included therein. For example, Isoglossinae are characterized as having two stamens, but plants of some genera placed in this subtribe by Lindau have four. Many of Lindau's taxa contain a core of genera that undoubtedly belong together, along with an odd assemblage of others. For example, his Diclipterinae include *Dicliptera* Juss., *Hypoestes* Sol. ex R. Br., *Periestes* Baill. (= *Hypoestes*), and *Peristrophe* Nees, a group readily recognized as cohesive by essentially all students of Acanthaceae. To this subtribe, however, Lindau added *Tetramerium* Nees. Plants of this last genus are morphologically more similar to *Carlowrightia* A. Gray and *Anisacanthus* Nees, which were placed by Lindau in Graptophylleae, than they are to other Diclipterinae. These problems have made it difficult for subsequent students of Acanthaceae to classify newly described genera with any confidence, such that Lindau's classification is not widely used.

Bremekamp (1965) outlined a revised infrafamilial classification of Acanthaceae, but did not always specify the generic composition of his tribes and subtribes. In a major realignment of taxa, he included six of the tribes placed by Lindau in Imbricatae (Asystasieae, Graptophylleae, Isoglosseae, Justicieae, Odontonemeae, Pseuderanthemeae) into an expanded Justicieae with three subtribes (Justiciinae, Rhytiglossinae, and Odontoneminae). Justiciinae and Rhytiglossinae (a substitute name for Lindau's Isoglossinae) were distinguished from Odontoneminae on the basis of an androecium of two stamens with no staminodes. Justiciinae were further distinguished by presence of a rugula (a channel in the upper lip of the corolla in which the style rests during anthesis), whereas plants with lenticular biporate pollen were assigned to Rhytiglossinae (= Isoglossinae). Most of the remaining Justicieae were placed in Odontoneminae. Plants belonging to this last group retain the plesiomorphic state of one or more of the characters that marked

MacClade, and two anonymous reviewers for insightful input on an earlier version of the manuscript. This research was partially supported by grants from the U.S. National Science Foundation to LAM (DEB BSR-8507517, DEB BSR-9707693), and from the University of Arizona small grants program. TFD's collecting activities were partially funded by the In-house Research Fund and Lindsay Field Research Fund of the California Academy of Sciences, the Oracle Corporation, the American Philosophical Society, and the Christensen Research Institute. The University of Arizona Research Training Group in the Analysis of Biological Diversification (NSF DIR-9113362, BIR-9602246) and the University of Arizona's Undergraduate Biological Research Participation program supported KMR.

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Bremekamp's other two subtribes, including absence of a rugula, androecium with four staminal elements (four fertile stamens or two stamens plus two staminodes), and pollen with colpoid streaks (= pseudocolpi). It is mostly feasible to assign newly described genera to Bremekamp's subtribes; however, Justiciinae and Odontoneminae, in particular, are very large groups of plants such that little is really accomplished by classifying to this level. Further, it is clear that although Justiciinae and Rhytiglossinae (= Isoglossinae) are likely marked by synapomorphies and may be monophyletic, Odontoneminae are not and are thus likely not monophyletic.

Problems in classifying Justicieae sensu Bremekamp (and as used herein) do not end at the subtribal level. The tribe includes a number of genera that are not clearly diagnosed as distinct from related genera (including *Justicia* with ca. 700 species; see Graham, 1988, who noted a conservative estimate of some 600 species at that time). This often makes routine identification difficult at best. In sum, there is a great deal to be learned about relationships in this richly diverse lineage of Acanthaceae.

Recent progress has affirmed the monophyletic status of Acanthaceae and advanced our understanding of relationships between Justicieae and the three other main lineages of Acanthaceae (McDade et al., 2000). Justicieae and Ruellieae sensu lato (s.l., i.e., sensu Manktelow et al., in press) are sister taxa. Barlerieae (including Whitfieldieae, see Manktelow et al., in press) are sister to these two together, and Acanthoideae (sensu Bremekamp, 1965) are sister to the first three together. The monophyly of each of these, as well as the pattern of relationships among them, is supported by molecular sequence data and, in some cases, by morphological synapomorphies (McDade & Moody, 1999; Manktelow et al., in press). There has, however, been little progress in understanding relationships within Justicieae. Based on admittedly sparse sampling, particularly of Old World plants, McDade and Moody (1999) and McDade et al. (2000) proposed at least five sublineages of Justicieae. Other authors, notably Daniel (1986), Daniel and Chuang (1993), and Daniel et al. (1984, 1990), have informally proposed that certain groups of genera are closely related, but these proposals have not been in the context of phylogenetic analyses nor have they been formalized.

Here we present a phylogenetic analysis based on molecular sequence data from two regions (the nuclear ribosomal ITS and chloroplast *trn*L-*trn*F spacer and intron) for a reasonably representative sample of Justicieae. We discuss these results in the context of evidence from other sources including morphology, chromosome numbers, and geographic distribution.

Materials and Methods

Taxon Sampling

We obtained sequences for species representing all of Lindau's (1895) suprageneric taxa of Imbricatae that were included by Bremekamp (1965) in his Justicieae. In addition, we attempted to sample two or more species of the larger genera of Justicieae, with emphasis on genera that occur in both the Old and New Worlds, e.g., *Justicia, Dicliptera.* From the very large genus *Justicia,* we included representatives of more than half of the sections recognized by Graham (1988). To root our hypothesis of relationships among Justicieae, we used two members of each of the other main lineages of Acanthaceae s. str. as outgroups: Ruellieae s.l. (one species each of *Ruellia* and *Sanchezia*), Barlerieae (one species each of *Barleria* and *Lepidagathis*), and Acanthoideae (one species each of *Aphelandra* and *Stenandrium*) (Appendix 1). We included the most distantly related groups within these lineages for which we had material and were able to obtain sequences. This should have the effect of "breaking" long branches (sensu Felsenstein, 1985) and thus increase confidence in our results (see Graybeal, 1998).

Molecular Methods

For most samples, DNA was extracted from fresh leaf material or material dried in silica gel; recently collected herbarium specimens were the source of DNA for the remaining species (Appendix 1). Total genomic DNA was extracted using the modified CTAB method of Doyle and Doyle (1987). Some acanths have pigmented compounds that apparently complex to DNA; this was dealt with by purifying the DNA, as described by McDade et al. (2000).

For the nr ITS sequences, a fragment comprising *its*1, the 5.8s gene, and *its*2 (Baldwin, 1992; Baldwin et al., 1995) was amplified. Early in this project, we used the "universal" primers "its4" and "its5" (Baldwin, 1992). As noted by McDade et al. (2000), some samples amplified with these primers yielded fungal contaminants. Using primers "C26A" and "N-nc18S10" designed for plants (Wen & Zimmer, 1996) effectively ended this problem. Optimal polymerase chain reaction (PCR) conditions to amplify double-stranded DNA varied somewhat among taxa, and we used a "touchdown" tem-

perature cycling profile to circumvent the process of optimizing PCR conditions for each taxon (see McDade et al., 2000). For the *trn*L-*trn*F sequences, a fragment comprising the *trn*L intron, the 3′*trn*L exon, and the intergenic spacer between this exon and the *trn*F gene of the chloroplast genome (Taberlet et al., 1991) was amplified using the "c" and "f" primers designed by these same authors. Standard PCR techniques were used to amplify double-stranded DNA.

Sequences were generated on ABI automated sequencers at the University of Arizona DNA sequencing facility using initially the same primers as in amplification. This yielded high quality sequences for essentially all of the cp *trn*L-*trn*F PCR templates and for about two-thirds of the nr ITS samples. However, the nr ITS region is extremely G-C rich in many Acanthaceae (see McDade et al., 2000) and poly-C or -G strings > 5 bp long are present in some taxa. DNA polymerase frequently was unable to read through these long repeats of Gs or Cs such that incomplete sequences were obtained. When only a partial *its*1 sequence was obtained using primer "N-nc18S10" (anchored in the 18s rDNA gene), we attempted to complete the sequence using primer "C26A" (anchored in the 26s rDNA gene). For some templates, neither the sequencing reaction primed with "N-nc18S10" nor that primed with "C26A" yielded a complete sequence. When this occurred, we sequenced using internal primers "its2" and "its3" (Baldwin, 1992), which are anchored in the 5.8s gene and yield sequence for *its*1 and *its*2, respectively. Both strands were sequenced except when sequencing with one primer yielded a complete sequence with no ambiguities (ca. ½ of the cp *trn*L-*trn*F templates and ¼ of nr ITS templates) or when sequencing with all available primers did not yield completely overlapping forward and reverse sequences (five of the nr ITS templates).

Electropherograms of all sequences were proofread manually. Overlapping portions were reconciled by reverse-complementing one, aligning the two, and double-checking any inconsistencies against the electropherograms; mismatches were coded as uncertain.

ALIGNMENT AND ANALYSES

Sequences were aligned separately by eye in SeqApp (Gilbert, 1994), and then moved into MacClade version 4.0a11 (Maddison & Maddison, 1999). These are available on request from LAM. As documented by McDade et al. (2000), the nr ITS region is quite divergent among Acanthaceae. Although alignments were straightforward within Justicieae, one region of *its*1 (aligned positions 65–204; ca. 15% of the total aligned length) could not be aligned with confidence between Justicieae and the six taxa used as outgroups. The six outgroup taxa were thus scored as missing for those nucleotide positions. Most of the total 3.5% missing data for the ingroup are in the highly conserved 5.8s gene. Most of the remaining missing data are in the *its*1 sequences for three taxa (*Fittonia* Coem., *Asystasia gangetica*, and *Spathacanthus parviflorus*); these sequences could not be completed despite attempts using all available primers. Alignment of the nr ITS sequences required introduction of many gaps, most of which were one or two bp in length, restricted to a single taxon, and in highly variable and/or G-C rich regions of the sequences. However, 14 indels were shared by two or more taxa, had concordant 5′ and 3′ termini, and did not overlap gaps in the sequences for other taxa. Information on these indels was added to the matrix as presence/absence characters.

The cp sequences were easily aligned across all taxa, including outgroups, despite the fact that, as noted by McDade and Moody (1999), this region is prone to length mutations. Seventeen parsimony informative indels were added to the matrix as presence/absence data. For the cp locus, 3.2% of data are missing; almost all of the missing data are in the relatively conserved 5′ end of the intron.

Data matrices were analyzed separately using PAUP* 4.0b2 (Swofford, 1999). All parsimony analyses were conducted using rigorous heuristic searches, i.e., 20 random addition sequences (all analyses found a single island sensu Maddison, 1991) and TBR swapping; gaps were treated as missing data. Multiple most parsimonious (MP) trees were combined as strict consensus trees.

In addition to standard measures of fit of characters to the resultant trees (consistency index, retention index), the strength of support for individual branches was estimated using bootstrap values (Felsenstein, 1985) and decay or Bremer indices (Bremer, 1988; Donoghue et al., 1992). For the nr ITS matrix, bootstrap (BS) values reported are from 200 full heuristic replicates with 20 random addition sequences and TBR branch swapping. The cp data provide poor resolution in some distal portions of the phylogeny such that most bootstrap replicates generated enough MP trees to swamp computer memory; it was thus not possible to use rigorous branch swapping methods. Instead, BS values are from 1000 replications with 500 random addition sequences each and no branch swapping. For both data sets, decay values for each branch were de-

Table 1. Characteristics of the nuclear ribosomal ITS region (59 taxa) and chloroplast *trn*L-*trn*F (52 taxa) in Justicieae (outgroup taxa not included in these calculations). Reporting of variable and parsimony informative sites includes sites within gaps, whereas sites within gaps were excluded for calculation of pairwise distances. [1] Includes 25 and 28 bp of the 18s and 26s ribosomal genes, respectively, that flank *its*1 and *its*2, plus the 5.8s gene. [2] Includes the 3′ *trn*F exon and 40 bp of the *trn*F gene that flanks the *trn*L-*trn*F spacer.

	*its*1	*its*2	nr ITS region	*trn*L3′ intron	Spacer	Intron + Spacer
Raw length	193–278	211–234	410–512	347–521	224–332	710–824
Aligned length	356	288	644 (860[1])	629	473	1103 (1191[2])
Variable sites (proportion)	209 (0.59)	176 (0.61)	421 (0.49)[1]	165 (0.26)	160 (0.34)	347 (0.31)[2]
Parsimony informative sites	175 (0.49)	114 (0.40)	308 (0.36)[1]	73 (0.12)	78 (0.16)	167 (0.15)[2]
Pairwise distances (range, %)	0.8–31.5%	0.5–33.2%	0.5–23.8%	0–9.6%	0–15.9%	0.4–10.9%[2]
GC content (range)	0.66–0.77	0.66–0.77	0.67–0.76	0.33–0.38	0.36–0.42	0.35–0.38[2]
Scored indels, number	12	2	14	7	10	17

termined by first using MacClade to prepare a set of trees each with a single branch resolved. These trees were then loaded into PAUP as constraint trees, and the program was asked to find the shortest trees inconsistent with the constraint tree. The difference between the length of these trees and the globally shortest trees is the decay index (DI) for the branch in question.

We obtained sequences for only one of the two loci for 13 taxa (see Appendix 1). Four of these (*Justicia brandegeana, J. spicigera, J. comata, Anisacanthus puberulus*) were sequenced for nr ITS but not the cp locus because sequences obtained earlier permitted us to judge that the more slowly evolving cp locus would be essentially invariant in these compared to close relatives (see McDade et al., 2000). DNA of the other taxa could not be amplified for the missing locus or more than ¼ of the sequence was missing even after attempts to sequence using all available primers. These taxa were included in the analysis of the locus for which complete (or nearly complete) data were available but were pruned from the data sets before combining. The data sets thus pruned to include complete sequences for the same set of 55 taxa (49 Justicieae + 6 outgroups) were combined into a single NEXUS file using the file editing capabilities of PAUP*. The nr ITS and cp *trn*L-*trn*F data sets were tested for congruence using Farris et al.'s (1995) Incongruence Length Difference test (implemented in PAUP* as the partition homogeneity test). Phylogenetic analyses of the combined data sets were conducted as described above; bootstrap values (200 replicates with 5 random addition sequences each) and decay indices (as previously described) were generated for each branch.

Alternative phylogenetic hypotheses were evaluated by using MacClade to prepare trees that reflect the relationships of interest. These were loaded into PAUP* as constraint trees, and the program was asked to find the shortest trees consistent with the topology in question. The difference between the length of these trees and the globally shortest trees provides an indication of the parsimony cost (in terms of additional evolutionary transitions) involved in accepting the alternative hypothesis.

To compare patterns of molecular evolution between loci and among lineages, matrices of pairwise HKY85 distances for both loci and for the combined sequences were output from PAUP* and moved into JMP (Sall & Lehman, 1996) for analysis. The relationship between pairwise distances for the two loci was examined using correlation. Rates of evolution were compared between selected sister lineages using a modified relative rates test (Sarich & Wilson, 1973). Distances between members of two sister lineages and their closest relative were tabulated, and *t*-tests were used to compare the means of these pairwise distances.

Results

Molecular evolution

Within the nr ITS region, *its*1 and *its*2 are roughly similar in variability except that *its*1 has more informative indels than *its*2 (Table 1). The cp *trn*L-*trn*F spacer is considerably more variable than the *trn*L3′ intron, and the spacer is likewise more prone to indels although this difference is not marked. Whether considered in terms of overall variable sites or parsimony informative sites, the nr ITS region is twice as variable among these taxa as the cp *trn*L-*trn*F region (Table 1). Similarly, maximum pairwise distances are more than twice as great for the nr ITS sequences as for the *trn*L-*trn*F data. Across all taxa, pairwise distances for the two loci are positively correlated ($r = 0.669$, $N = 1176$, $P < 0.0001$), suggesting that although the two loci

are evolving at different rates, those rates are fairly consistent across Justicieae. Variation in rates of evolution among lineages will be discussed below in the context of phylogenetic relationships.

PHYLOGENETIC RELATIONSHIPS

Results of the partition homogeneity test indicate that the two data sets are not incongruent (P = 0.34). Further, except as regards taxa for which sequence data from only one locus were available, the topology obtained from the combined data set (Fig. 1) differs from the trees obtained from the separate data sets (not shown) only in degree of resolution or in weakly supported portions. For example, the nr ITS data do not resolve *Ptyssiglottis* T. Anderson as part of Isoglossinae, but do not support any other placement of this taxon. Similarly, the cp *trn*L-*trn*F data do not resolve relationships among most species of Old World *Justicia*, whereas the nr ITS data provided a fully (but weakly) supported hypothesis of relationships among these taxa. As expected, given congruence of the data sets and increased number of characters in the combined data set [308 and 218 parsimony informative characters in the nr ITS and cp *trn*L-*trn*F data sets, respectively, for a total of 526 in the combined data set (note that these tallies of parsimony informative characters are from the data sets pruned to include taxa for which both sequences were available)], branch support increases markedly in the combined topology. For all of these reasons, discussion of relationships is based on the outcome of the combined analysis.

Figure 1 presents the strict consensus of most parsimonious (MP) trees produced by analysis of the combined data sets. Note that only major lineages within Justicieae are labeled to emphasize higher level patterns of relationship; Figures 2 and 3 provide detail on relationships within lineages. In our discussion of relationships, when lineages identified here largely conform to previously recognized taxa, we use names of these established taxa; when lineages have not been previously recognized or named, we use informal names.

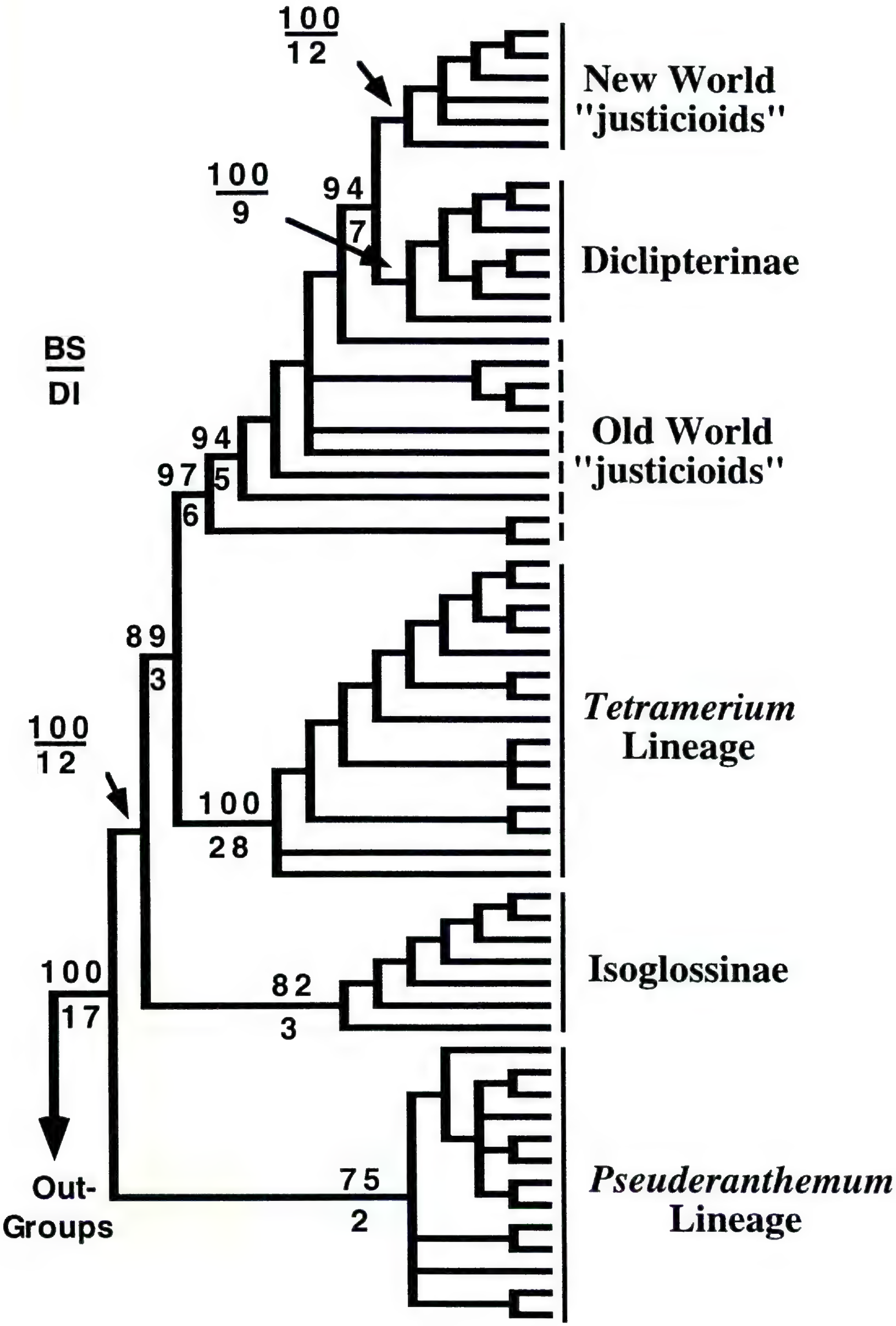
The combined analysis provides strong support for monophyly of Justicieae (Fig. 1, BS = 100, DI = 17). Within Justicieae, five lineages are moderately, e.g., the *Pseuderanthemum* lineage, BS = 75, DI = 2, to very strongly, e.g., the *Tetramerium* lineage, BS = 100, DI = 28, supported as monophyletic. The *Pseuderanthemum* lineage is sister to all other Justicieae, which are strongly supported as monophyletic (BS = 100, DI = 12). Isoglossinae are moderately well supported as monophyletic (BS = 82, DI = 3), and as sister to all Justicieae excluding the *Pseuderanthemum* lineage (BS = 89, DI = 3). The *Tetramerium* lineage is very strongly supported as monophyletic; this group is sister to a monophyletic lineage that includes Diclipterinae and *Justicia* and close relatives. These taxa, i.e., all Justicieae above the *Tetramerium* lineage exclusive of Diclipterinae and inclusive of both Old and New World plants, will be referred to subsequently as "justicioids." The labels *New World "justicioids"* and *Old World "justicioids"* will be used to refer to geographically delimited assemblages. The "justicioids" and Diclipterinae lineage is strongly supported as monophyletic (BS = 97, CI = 6). There is strong support for monophyly of the New World "justicioid" lineage (BS = 100, DI = 12) and of Diclipterinae (BS = 100, DI = 9), and for the sister-group relationship of these lineages (BS = 94, DI = 7). However, Old World "justicioids" are placed as a paraphyletic assemblage below New World "justicioids" + Diclipterinae.

Figures 2 and 3 are strict consensus trees showing placement of all included taxa, and bootstrap and decay support for all branches. Taxa for which sequence data for only one locus was available have been added to Figures 2 and 3 using stylistic conventions, i.e., branches are angled and taxon labels and support values are indicated in smaller type, to signal that the result is based on partial data. One randomly chosen MP tree is presented as Figure 4 to illustrate branch lengths.

Pseuderanthemum *lineage.* There is only modest support for monophyly of this lineage from the combined analysis (BS = 75, DI = 2). Nr ITS data indicate that *Spathacanthus* Baill. belongs here, and that the two included species are each other's closest relatives with strong support (Fig. 2; BS = 89, DI = 4). Our data do not resolve relationships

→

Figure 1. Major patterns of relationships among Justicieae from parsimony analysis of combined nr ITS and cp *trn*L-*trn*F sequence data. Strict consensus of 24 most parsimonious trees (length = 2455, CI = 0.557, RI = 0.690). Bootstrap and decay indices are presented for major lineages only (see Figs. 2 and 3 for details of relationships within lineages and support for these). Note that all labeled lineages are monophyletic (indicated by solid vertical lines) with the exception of Old World "justicioids," which are a grade (indicated by dashed vertical line), as discussed in the text.


100
12
100
9
94
7
BS
DI
94
97
5
6
89
3
100
12
100
28
100
17
82
3
75
2
Out-
Groups
New World
"justicioids"
Diclipterinae
Old World
"justicioids"
Tetramerium
Lineage
Isoglossinae
Pseuderanthemum
Lineage

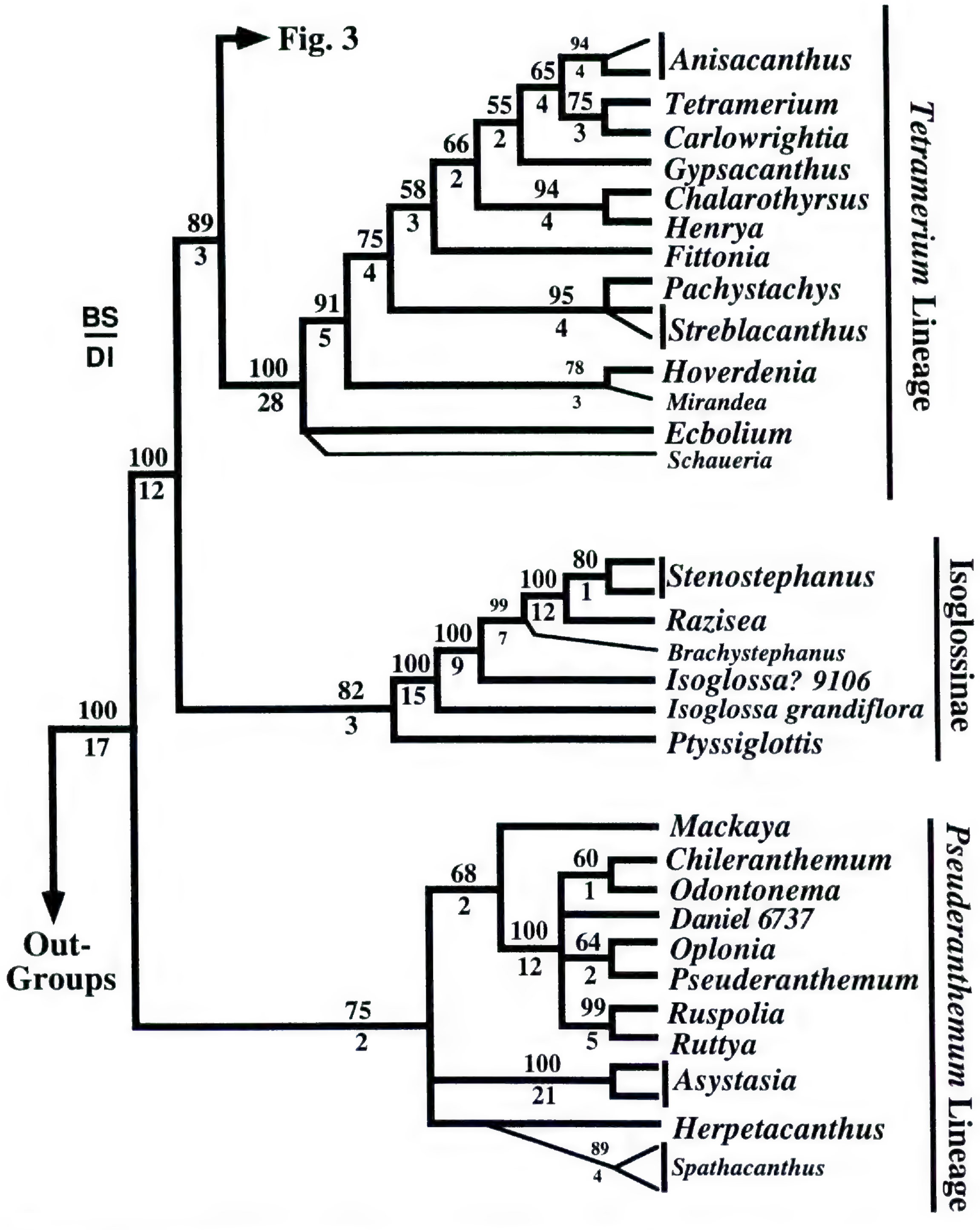


Figure 2. Relationships among members of the *Pseuderanthemum* lineage, Isoglossinae, and the *Tetramerium* lineage. Vertical lines link congeneric species when these are monophyletic. Taxa with bold, large typeface labels on thick branches are in the combined analysis (nr ITS + cp *trn*L-*trn*F); taxa for which data for only the nr ITS was available are added on thinner, angled branches with smaller typeface labels. Data for both loci were obtained for *Anisacanthus thurberi* and *Streblacanthus cordatus*, whereas only nr ITS data were obtained for a second member of each genus (*A. puberulus* and *S. roseus*; see Appendix 1). Bold, large typeface bootstrap and decay values are from the combined analysis; those in smaller typeface are from the analysis of the nr ITS data alone.

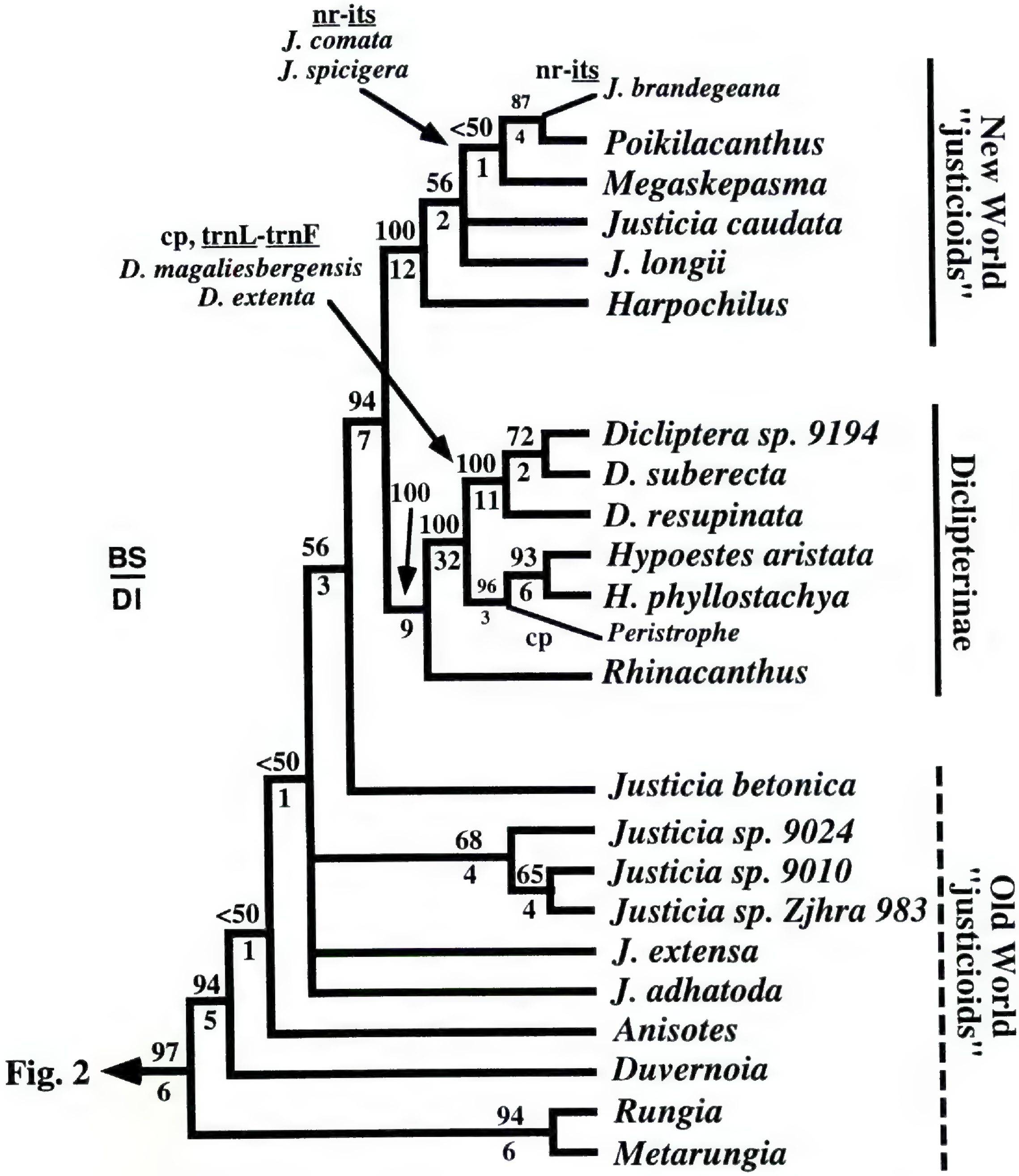


Figure 3. Relationships among Old World "justicioids," Diclipterinae, and the New World "justicioid" lineage. Taxa with bold, large typeface labels on thick branches are in the combined analysis (i.e., nr ITS + cp *trn*L-*trn*F); taxa for which data for only one locus was available are added on thinner, angled branches or to the upper left. Bold, large typeface bootstrap and decay values are from the combined analysis; those in smaller typeface are from separate analyses of either the nr ITS or cp *trn*L-*trn*F data set. The positions of *Justicia brandegeana* (nr ITS) and *Peristrophe* (cp *trn*L-*trn*F) are resolved and strongly supported, whereas *J. comata* and *J. spicigera* (nr ITS), *Dicliptera extenta* and *D. magaliesbergensis* (cp *trn*L-*trn*F) are placed in polytomies with other New World *Justicia* and with *Dicliptera*, respectively.

among basal groups in the lineage [i.e., *Spathacanthus* (nr ITS only), *Herpetacanthus* Nees, *Asystasia* Blume]. The two representatives of *Asystasia* are, however, strongly supported as monophyletic; these species also share two unique indels, one in each genic region. Further, there is considerable molecular divergence between the two *Asystasia* species, and between *Asystasia* and other members of the

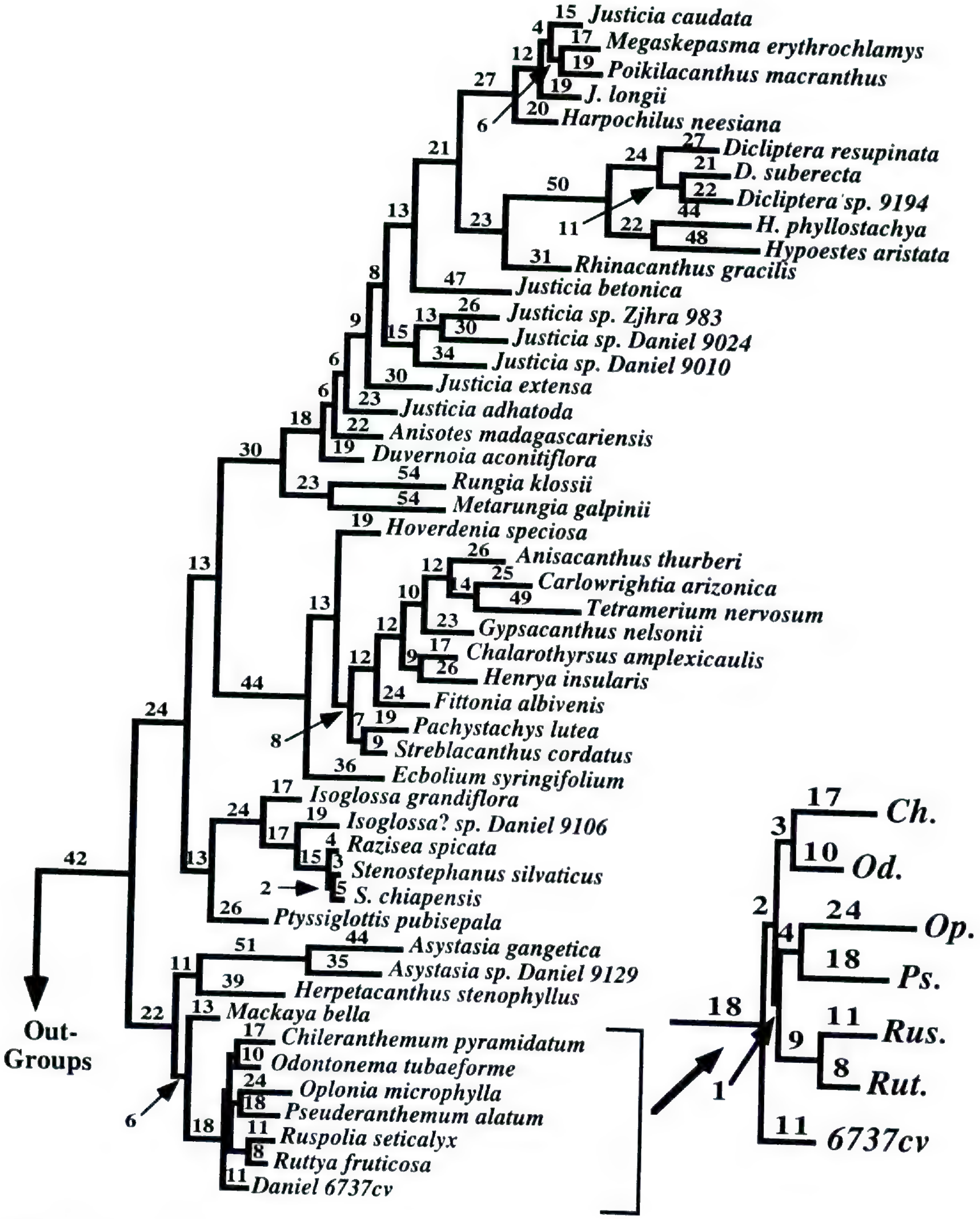


Figure 4. One (randomly chosen) of the most parsimonious trees from analysis of the nr ITS and cp *trn*L-*trn*F combined sequence data for all taxa for which data for both loci were available. Branch lengths are proportional to number of changes using ACCTRAN optimization; numbers on branches report branch length.

Pseuderanthemum lineage (note branch lengths in Fig. 4). Above this unresolved group of basal genera, there is weak support for *Mackaya* Harv. as sister to all others (BS = 68, DI = 2), and strong support for monophyly of the lineage above *Mackaya* (BS = 100, DI = 12). This last group includes one Malagasy species (*Daniel 6737cv*) that corresponds to specimens at P that were annotated as "*Justicia petiti* Benoist." It does not, however, appear that Benoist ever formally described this tax-

on, and it remains either unnamed or described with another name. There is little molecular divergence within the lineage above *Mackaya* (note short branch lengths, Fig. 4), and only the sister taxa *Ruspolia* Lindau + *Ruttya* Harv. are resolved with strong support (BS = 99, DI = 5).

Isoglossinae. A lineage that largely conforms to Lindau's (1895) Isoglossinae is identified by the combined analysis as monophyletic with modest support (BS = 82, DI = 3). The group of genera above *Ptyssiglottis* is extremely strongly supported as monophyletic (BS = 100, DI = 15). The Old World genus *Isoglossa* Oerst. may not be monophyletic: a putative species of *Isoglossa* (i.e., *Daniel 9106*) is more closely related to *Stenostephanus* Nees, *Razisea* Oerst., and *Brachystephanus* Nees (BS = 100, DI = 9) than to *I. grandiflora*. The nr ITS data place Old World *Brachystephanus* with New World *Razisea* + *Stenostephanus*, with strong support (BS = 99, DI = 7; note that these support values are from the nr ITS analysis alone). The two species of *Stenostephanus* are placed together, but with only modest support (BS = 80, DI = 1). There is, however, strong support for monophyly of *Razisea* + *Stenostephanus* (BS = 100, DI = 12). Short branch lengths among *Razisea* and *Stenostephanus* (Fig. 4) indicate that there is little molecular divergence among these three species.

Tetramerium *lineage.* The *Tetramerium* lineage is extremely strongly supported as monophyletic (BS = 100, DI = 28). Nr ITS data indicate that *Schaueria* Nees is part of this lineage but do not resolve its relationships. Further, the nr ITS data place the two sampled species of *Streblacanthus* Kuntze as sister taxa, and also place the two sampled species of *Anisacanthus* together (cp data not available for *S. roseus* and *A. puberulus*). There is strong support for monophyly of a lineage above *Ecbolium* Kurz (and *Schaueria*, nr ITS data only) (BS = 91, DI = 5), and moderate support for the lineage above *Hoverdenia* Nees (and *Mirandea* Rzed., nr ITS data only) (BS = 75, DI = 4). The combined data set provides strong support for sister-group relationships of *Streblacanthus* + *Pachystachys* Nees (BS = 95, DI = 5) and *Chalarothyrsus* Lindau + *Henrya* Nees ex Benth. (BS = 94, DI = 4), and nr ITS data provide moderate support for *Hoverdenia* + *Mirandea* (BS = 78, DI = 3; note that these values are from the nr ITS data alone). Although there is weak support for the internal nodes of the phylogeny distal to *Pachystachys* + *Streblacanthus*, monophyly of a lineage including *Anisacanthus*, *Tetramerium*, *Carlowrightia*, and *Gypsacanthus* E. J. Lott, V. Jaram. & Rzed. seems highly likely based on a shared deletion in the spacer region of *trn*L-*trn*F that is ca. 150 bp in length.

"*Justicioids*" *and Diclipterinae.* There is strong support from the combined analysis (Fig. 3) for a clade comprised of much of Lindau's (1895) Diclipterinae plus all included "justicioids" (i.e., species of *Justicia* and the allied genera *Rungia* Nees, *Metarungia* Baden, *Duvernoia* E. Mey. ex Nees, *Anisotes* Nees from the Old World; *Harpochilus* Nees, *Megaskepasma* Lindau, and *Poikilacanthus* Lindau from the New World) (BS = 97, DI = 6). Within this lineage, the representatives of *Justicia* are not monophyletic. Instead, the Old World species are a paraphyletic assemblage that is basal to the Diclipterinae (as here circumscribed, see below) + the New World "justicioids" lineage. New World *Justicia* are monophyletic only if *Poikilacanthus* and *Megaskepasma* are included. Old World genera traditionally aligned with *Justicia* are placed as a series of lineages basal to Old World *Justicia*, with strong support for *Rungia* + *Metarungia* (BS = 94, DI = 6), for monophyly of all others above this pair (BS = 94, DI = 5), and then weak support for precise placement of *Duvernoia*, *Anisotes*, and the Old World representatives of *Justicia*.

There is strong support for monophyly of the Diclipterinae + New World "justicioids" lineage (BS = 94, DI = 7), and very strong support for monophyly of each of these lineages (Fig. 3). The lineage referred to here as Diclipterinae largely conforms to the core of Lindau's taxon of that name. *Rhinacanthus* Nees (not part of Lindau's Diclipterinae) is strongly supported as a basal member of the lineage, with very strong support for monophyly of all included taxa above *Rhinacanthus* (BS = 100, DI = 32). *Hypoestes* is monophyletic with strong support (BS = 93, DI = 6), and the cp *trn*L-*trn*F data place *Peristrophe* as sister to *Hypoestes*. The genus *Dicliptera* is monophyletic, including both Old and New World members. Especially above *Rhinacanthus*, branch lengths are notably longer in Diclipterinae than in adjacent groups (Fig. 4). Using *Justicia betonica* as the outgroup, a relative rates test indicates that sequence divergence in Diclipterinae is higher than in its sister group (the New World "justicioid" lineage). This is true for the loci considered separately (nr ITS: t = 3.229, 13 df, P = 0.0066; cp *trn*L-*trn*F: t = 3.89, 12 df, P = 0.0021) as well as for the combined data (t = 3.838, 9 df, P = 0.004).

New World "justicioids" (i.e., representatives of *Justicia* plus *Harpochilus*, *Megaskepasma*, and *Poikilacanthus*) are together monophyletic, with strong support (Fig. 3, BS = 100, DI = 12). Nr ITS sequence data place *J. brandegeana* as sister to *Po-*

ikilacanthus with strong support (BS = 87, DI = 4, nr ITS data alone), and place *J. comata* and *J. spicigera* as part of the clade that includes all other New World *Justicia*, but without resolution. Figure 4 indicates that branch lengths are quite short among members of the New World "justicioid" lineage, and a relative rates test shows lower levels of sequence divergence in this clade compared to its sister group, Diclipterinae.

Discussion

Justicieae are strongly supported as monophyletic in our analysis, and this has been confirmed in other analyses with richer samples of other Acanthaceae (McDade & Moody, 1999; McDade et al., 2000). This result is supported by nucleotide substitutions as well as by indels: all Justicieae included here share three indels in the cp locus. Unusual tricolporate hexapseudocolpate pollen grains (see figs. 7–10 in Daniel, 1998a) occur in all lineages of Justicieae as here delimited (although shifts to other types of grains are synapomorphies for some clades as discussed below). Pollen grains of this type are not known among Acanthaceae outside of Justicieae and, to our knowledge, are not known in other angiosperms. We here propose this character as a synapomorphy for Justicieae. Bremekamp (1965) proposed that this pollen type characterizes plants of his subtribe Odontoneminae; it is thus not surprising that his taxon is not monophyletic but instead assembles a heterogeneous group of Justicieae that lack more derived pollen types.

Lack of hygroscopic trichomes on the seeds characterizes Justicieae but may not be a synapomorphy. Seeds of plants of Ruellieae s.l., the sister group of Justicieae (see McDade et al., 2000), have hygroscopic trichomes (exceptions are genera previously included in Louteridieae and Trichantereae, which have apparently lost these structures). Seeds of plants of Barlerieae + Whitfieldieae (these together comprise the sister group of Justicieae + Ruellieae s.l.; see Manktelow et al., in press) also have hygroscopic trichomes, again with a few exceptions in which they have apparently been lost secondarily. However, there is evidence that the hygroscopic trichomes differ between Ruellieae s.l. and Barlerieae + Whitfieldieae such that they are not likely homologous (Grubert, 1974; Scotland et al., 1995; Manktelow, 1996). If this is the case, then the common ancestor of these lineages would have lacked hygroscopic trichomes. Regardless of the phylogenetic status of the character, lack of hygroscopic trichomes distinguishes Justicieae from plants of the other two lineages, with just a few exceptions. Acanthoideae also lack hygroscopic trichomes on the seeds, but these plants lack also the synapomorphies that link the other three lineages of Acanthaceae s. str., i.e., Ruellieae s.l., Barlerieae, Justicieae (e.g., cystoliths, articulated stems, colporate or porate pollen) and are thus unlikely to be confused with them.

Pseuderanthemum *lineage.* The position of these taxa as basal within Justicieae is clear from our data, but there is only modest support for their monophyly. Further, we know of no morphological synapomorphies for the entire lineage. Compared to other Justicieae, these plants have an androecium of four staminal elements (all four may be fertile or two may be reduced to staminodes). However, this is no doubt plesiomorphic for Justicieae: it is hypothesized to be a synapomorphy for all Lamiales s.l. (i.e., sensu Olmstead et al., 1993), and the other major lineages of Acanthaceae include taxa with this trait. Additional data will be necessary to test whether the lineage is indeed monophyletic or instead a series of lineages at the base of Justicieae. Most aspects of relationships within the *Pseuderanthemum* lineage are not resolved with confidence by our data. There is little divergence among most of these taxa for the loci examined here, and it will be necessary to work with morphological characters or more rapidly evolving loci to make progress in unraveling these phylogenetic relationships.

There is some corroborating evidence for those aspects of relationships within the *Pseuderanthemum* lineage that do emerge from our analysis. Whereas plants placed in unresolved fashion at the base of the lineage have four fertile stamens, two of these are reduced to staminodes in plants of the *Mackaya–Ruttya* clade. Further, although chromosome numbers have not been determined for *Chileranthemum* Oerst. or for the unidentified Malagasy taxon represented by *Daniel 6737cv*, all other genera in the *Mackaya–Ruttya* clade appear to have a base chromosome number of $x = 21$ (Daniel & Chuang, 1989; Daniel et al., 1990; Daniel & Chuang, 1993, 1998). The only chromosome number known for *Mackaya* (Old World, 2 species), $n = 42$, is from a single count by Daniel and Chuang (1989); $n = 42$ has not been recorded for any other genus in the lineage. A chromosome complement of $n = 21$ has been infrequently reported in genera representing various other lineages within the family, but does not seem to characterize other large, suprageneric groups. Below the *Mackaya–Ruttya* clade in the *Pseuderanthemum* lineage, chromosome counts are not available for *Herpetacanthus* (New World, ca. 10 species) or *Spathacanthus* (Neo-

tropical, 3 species), and $x = 21$ may thus be a synapomorphy for a more inclusive lineage. Interestingly, *Asystasia* (Old World, ca. 50 species) has $x = 13$ and includes species with polyploid derivatives of that number (Daniel, 2000). Several authors have suggested that $x = 7$ is symplesiomorphic for Acanthaceae (Grant, 1955; Raven, 1975; Piovano & Bernardello, 1991; Daniel & Chuang, 1993). If so, $x = 13$ has evolved via both polyploidy and dysploidy, and $x = 21$ may represent a hexaploid derivative of this base number.

There is very strong molecular support for the *Chileranthemum–Ruttya* clade but, within that clade, the only strongly supported relationship is that of *Ruspolia* and *Ruttya* as sister taxa. These two genera differ from others in the *Chileranthemum–Ruttya* clade in having monothecous stamens. *Ruspolia* (Africa and Madagascar, 4 species) and *Ruttya* (Africa, 3 species) are clearly closely related based on both molecular and morphological evidence, and a natural intergeneric hybrid, ×*Ruttyruspolia* A. Meeuse & de Wet, is known between them.

Heterostyly has been reported or observed by TFD or LAM in all genera of the *Chileranthemum–Ruttya* clade except *Ruttya* (based on the limited material available, it is not possible to determine whether *Daniel 6737cv* was collected from a heterostylous population). Given that this trait has only recently been observed in *Ruspolia* (LAM, pers. obs.), its absence in *Ruttya* may reflect lack of study rather than absence of the character. Heterostyly is otherwise unknown among Acanthaceae [the stylar polymorphism reported by Long (1971) for *Ruellia caroliniensis* (Walter) Steud. is related to corolla size but not to anther position]. As noted by Daniel (1995a), distinctions among *Chileranthemum* (New World, 3 species), *Odontonema* Nees (New World, ca. 30 species), *Oplonia* Raf. (American tropics and Madagascar, 14 species), and *Pseuderanthemum* Radlk. (pantropical, ca. 60 species) pertain primarily to differences in form of the corolla, which likely represent adaptations for different pollinators. These traits are well known to vary at low taxonomic level in Acanthaceae (e.g., Ezcurra, 1993, for *Ruellia*; Graham, 1988, for *Justicia*). We know of no diagnostic morphological features for these genera, and the monophyly of each should be tested.

Based on both molecular and morphological data, it seems clear that *Daniel 6737cv* should not be placed in *Justicia*; like other plants in the *Pseuderanthemum* lineage, it has an androecium of four staminal elements (two stamens plus two staminodes in this case, in contrast to *Justicia*, which lacks staminodes), and tricolporate hexapseudocolpate pollen (which is rare in *Justicia*). Like many Malagasy plants, its taxonomic placement requires additional study; our results do not indicate a clear generic assignment for the species.

The *Pseuderanthemum* lineage is a taxonomically heterogeneous group, including representatives of Lindau's (1895) tribes Asystasieae (*Asystasia*, *Spathacanthus*), Graptophylleae (*Ruspolia*), Isoglosseae (*Herpetacanthus*), Odontonemeae [*Chileranthemum*, *Mackaya*, *Odontonema*, *Ruttya*, and *Oplonia* (as *Anthacanthus* Nees)], and Pseuderanthemeae (*Pseuderanthemum*). To complete our understanding of this lineage and to test its monophyletic status, it will be important to include representatives of all Justicieae that have four fertile stamens or two stamens and two staminodes. As will be clear from his classification of the genera included here, Lindau (1895) distributed plants with four staminal elements in a number of tribes including Asystasieae (e.g., *Asystasiella* Lindau, *Chameranthemum* Nees, and *Thomandersia* Baill. in addition to those already included here), Graptophylleae (*Graptophyllum* Nees), Isoglosseae (*Podorungia* Baill., *Chlamydacanthus* Lindau, *Forcipella* Baill.), Odontonemeae (*Ballochia* Balf. f., *Phialacanthus* Benth., *Filetia* Miq.), and Pseuderanthemeae (*Codonacanthus* Nees). Many of these genera have not been studied in the century since Lindau's (1895) classification was proposed. More recently described genera with four staminal elements include *Pranceacanthus* Wassh. and *Pulchranthus* V. M. Baum, Reveal & Nowicke. Based on our results we predict that plants in most of these genera will be placed either as part of a monophyletic *Pseuderanthemum* lineage or as a series of basal lineages within Justicieae. It would also be useful to test monophyly of some of the larger genera in this lineage, especially those that are pantropical in distribution (*Pseuderanthemum*) or that have disjunct ranges (*Oplonia*, with 5 Malagasy and 9 New World species). Given the uncertainties noted above regarding exact taxonomic composition, we estimate that the *Pseuderanthemum* lineage comprises about 200 species.

As noted above, whether or not the *Pseuderanthemum* lineage is monophyletic, there is very strong support from our analysis for monophyly of all other Justicieae. These plants have an androecium of two stamens; that is, with only two exceptions of which we are aware, they have lost even staminodial remnants of the other pair. Remarkably, plants of *Chalarothyrsus* (here placed as a member of the *Tetramerium* lineage, see below) have four dithecous stamens. Two species of *Ptyssiglottis*

(here placed as the basal member of Isoglossinae, see below) are reported to have small or remnant staminodes (Hansen, 1992).

Isoglossinae. Lindau's (1895) key to tribes and subtribes distinguishes Isoglossinae based on the presence of two stamens with mono- or dithecous anthers and "Gürtelpollen" (i.e., girdled pollen). However, in describing the subtribe and in assigning genera to it, he included also some plants that lack this pollen type and have four stamens (e.g., *Herpetacanthus*, *Populina* Baill.). As discussed below, our results suggest that Lindau was correct in recognizing a group marked by girdled pollen. He was also correct in assigning additional genera that lack this synapomorphy to Isoglossinae, but erred in at least some assignments, e.g., *Herpetacanthus* is a member of the *Pseuderanthemum* lineage.

In our analysis, *Ptyssiglottis* (southeastern Asia to Papuasia, 33 species) is placed in Isoglossinae with moderate support. These plants have dithecous anthers and a diversity of pollen types including the basic type for Justicieae (tricolporate hexapseudocolpate pollen), but not the girdled biporate pollen characteristic of "core Isoglossinae" (Hansen, 1992; see below). *Ptyssiglottis* was placed by Lindau (1895) in Pseuderanthemeae, probably on the basis of its pollen. As delimited by Hansen (1992) plants of *Ptyssiglottis* lack staminodia with the two exceptions noted above of small or remnant staminodia. We are, however, unable to point to clear, non-molecular synapomorphies linking this genus to other Isoglossinae. Hansen (1992) treated *Ptyssiglottis* as a member of Isoglossinae but did not describe synapomorphies supporting the relationship. It would be well to test the present hypothesis that *Ptyssiglottis* is part of Isoglossinae by additional data (DNA sequence or otherwise). If this is the case, then the genus can be viewed as transitional between more typical Justicieae and "core Isoglossinae," which are marked by distinctive pollen morphological synapomorphies.

"Core" Isoglossinae (i.e., *Isoglossa* through *Stenostephanus*) are extremely strongly supported as monophyletic in our analysis. In addition to sequence data, this clade is marked by three unique and unreversed indels (two in the nr ITS locus). These plants also appear to share Lindau's "Gürtelpollen," which is typically biporate with pores that are surrounded by a more or less circular region. The two circular regions are separated from one another by a peripheral band (continuous or interrupted) of varying width. This type of pollen is described and figured by Daniel (1999) for *Stenostephanus* and *Razisea*. Similar pollen has been described in *Brachystephanus* (Figueiredo & Keith-Lucas, 1996) and *Isoglossa* (Muller et al., 1989; Raj, 1961, as *Rhytiglossa* Nees ex Lindl.), although at least the former genus shows more diversity in features such as aperture number. The identity of *Daniel 9106* from Madagascar has yet to be determined, but preliminary examination of its pollen reveals biporate pollen like that of other "core" Isoglossinae, and its macromorphological characteristics suggest its placement in *Isoglossa*. If it indeed represents a species of this genus, then the two species of *Isoglossa* included in our analysis do not form a monophyletic group. Instead, *Daniel 9106* is more closely related to *Stenostephanus*, *Razisea*, and *Brachystephanus* than to *I. grandiflora*. Interestingly, in addition to sequence support for this relationship, *Daniel 9106* also shares two indels in the *trn*L-*trn*F region with *Stenostephanus* + *Razisea* that *I. grandiflora* lacks. The nr ITS data support monophyly of *Brachystephanus* (tropical Africa and Madagascar, 13 species) and Neotropical *Stenostephanus* (ca. 75 species) and *Razisea* (4 species). These three genera share monothecous anthers as a morphological synapomorphy. The New World members of this sublineage have been divided into a series of small genera reflecting remarkable floral diversity (e.g., *Cylindrosolenium* Lindau, *Kalbreyeracanthus* Wassh., *Kalbreyeriella* Lindau). Wood (1988) treated most of these as congeneric with *Habracanthus* Nees, and Daniel (1995b, 1999) further combined *Habracanthus* with *Stenostephanus*. Nomenclatural changes are gradually being made to reflect this recasting of generic limits, e.g., Wasshausen (1999). Our results indicate that there is considerable merit to this approach and confirm that *Razisea* is part of this group as well (Daniel, 1999). However, investigation of relationships among the New World species using molecular data will require a locus evolving more quickly than those we have studied (note short branch lengths in Fig. 4).

Isoglossinae, as here defined, are particularly poorly known cytologically. Among genera studied by us, chromosome counts have been determined for only five species. It is noteworthy that the Neotropical genera *Stenostephanus* and *Razisea* appear to share a common chromosome number of $n = 18$ (Daniel, 1999). No chromosome numbers have been reported for the Old World genera *Brachystephanus* or *Ptyssiglottis*, and only a single count ($n = 17$) has been reported for the Old World genus *Isoglossa* (widespread in Old World tropics and subtropics, ca. 60 species) (Daniel & Chuang, 1998).

To delimit Isoglossinae clearly, it would be well to include representatives of other genera that lack the synapomorphies that mark "core" Isoglossinae.

Genera that were included in this subtribe by Lindau (1895, 1897) and that would appear to be part of "core" Isoglossinae as defined here include *Cylindrosolenium*, *Populina*, and *Oreacanthus* Benth. These, and other more recently described genera with two dithecous stamens and "Gürtelpollen" (e.g., *Conocalyx* Benoist, *Sphacanthus* Benoist), should be added. Acknowledging uncertainty about placement of some of these smaller genera, we estimate that Isoglossinae include about 200 species.

Monophyly of the larger genera of Isoglossinae should be tested, including *Ptyssiglottis*, *Isoglossa*, and *Stenostephanus*. Monophyly of *Brachystephanus* should be tested, and the usefulness of maintaining this genus separate from *Stenostephanus* also merits evaluation. Broad-scale studies of a number of taxa have demonstrated that morphologically similar genera in widely separated geographic regions are often congeneric (e.g., *Stenandrium* Nees–*Stenandriopsis* S. Moore, Vollesen, 1992; *Oplonia–Forsythiopsis* Baker, Stearn, 1971; *Mendoncia* Vell. ex Vand., *Afromendoncia* Gilg ex Lindau, and *Monachochlamys* Baker, Benoist, 1925).

Monophyly of all Justicieae above these first two lineages (i.e., *Pseuderanthemum* lineage and Isoglossinae, Fig. 1) is moderately supported (BS = 89, DI = 3), but we know of no non-molecular synapomorphies that mark this lineage.

Tetramerium *lineage.* Sequence data strongly support monophyly of this lineage; indeed, it is one of the most strongly supported clades in all of Justicieae. In addition to sequence data, all of the plants included in our analysis uniquely share five indels in the nr ITS region. The genera sampled here were placed by Lindau (1895) in diverse tribes: Asystasieae (*Chalarothyrsus*, part of *Henrya* [as *Solenoruellia* Baill.]), Graptophylleae (*Anisacanthus*, *Carlowrightia*, *Pachystachys*), Isoglosseae (*Fittonia*), and Odontonemeae [*Ecbolium*, *Hoverdenia*, *Schaueria*, *Streblacanthus*, *Tetramerium* (including *Henrya*)]. Despite Lindau's treatment and the fact that we can identify no clear non-molecular synapomorphies for the lineage, a group corresponding closely to that delimited here has been consistently identified based on monographic and cytological studies (Daniel, 1986, 1990; Daniel & Chuang, 1993; Daniel et al., 1984). A chromosome number of $n = 18$ seems to characterize the entire group, with additional diversity in some genera [e.g., *Ecbolium linneanum* has been reported to have $n = 14$ (one count), 18 (six counts), and 20 (one count); chromosome data are lacking for *Hoverdenia* and *Schaueria*]. Because $n = 18$ also occurs in Isoglossinae and in a few species of *Justicia*, it is difficult to determine whether it is a synapomorphy for the *Tetramerium* lineage or for a more inclusive group. These plants share a number of traits that are symplesiomorphic at this level within Justicieae: tricolporate hexapseudocolpate pollen (a synapomorphy for all Justicieae); an androecium of two bithecous stamens and no staminodes (a synapomorphy for Justicieae above the *Pseuderanthemum* lineage); and thecae that are parallel, inserted at more or less the same height on the filament, and unappendaged (these thecal characteristics are variously modified in many members of the "justicioids" and Diclipterinae lineage).

The placement of *Chalarothyrsus* within the *Tetramerium* lineage is surprising in that these plants have four bithecous stamens, a trait known to occur among Justicieae only in the *Pseuderanthemum* lineage, as here defined. However, *Chalarothyrsus* has a chromosome complement of $n = 18$ (contra $n = 21$, which characterizes the *Pseuderanthemum* lineage, see above). We can only conclude that there was a reversal to four functional stamens in the evolutionary history of plants of this unispecific genus from western Mexico. Given that occasional flowers with atypical androecia have been observed in a number of species of Acanthaceae (TFD & LAM, pers. obs.), such a reversal does not seem especially improbable. Further, trees placing *Chalarothyrsus* with the *Pseuderanthemum* lineage are 68 steps (3%) longer than the MP trees.

Within the *Tetramerium* lineage, most aspects of relationships are not strongly supported. Monophyly of all included genera above *Ecbolium* (Old World, 22 species) [+ *Schaueria* (New World, ca. 10 species), nr ITS data only] is strongly supported, and that of *Anisacanthus* through *Streblacanthus* is moderately well supported. We are not able to identify clear non-molecular synapomorphies for these lineages. There is moderate to strong support for sister relationships between *Hoverdenia* (Mexico, 1 species) + *Mirandea* (Mexico, 4 species) (nr ITS only), *Pachystachys* (West Indies and South America, 12 species) + *Streblacanthus* (Central and South America, 4 species), and *Chalarothyrsus* + *Henrya* (North and Central America, 2 species) but, again, non-molecular evidence is lacking. A clade comprised of *Anisacanthus* through *Henrya* (Fig. 2) is not especially strongly supported by sequence data (BS = 66, DI = 2), but is marked by a unique 22 bp deletion in the nr ITS region. Similarly, a clade composed of the New World genera *Anisacanthus* (ca. 20 species), *Tetramerium* (28 species), *Carlowrightia* (24 species), and *Gypsacanthus* (1 species) is only weakly supported by sequence data but, as noted above, these plants share a > 150bp deletion in the *trn*L-*trn*F spacer. This shared de-

letion argues strongly for their monophyly and also explains the weak support from sequence data: the cp region that is absent in these four genera is the most variable of the cp locus (i.e., the most likely source of nucleotide substitutions among close relatives). The paucity of morphological evidence for relationships in the *Tetramerium* lineage reflects Daniel and Chuang's (1993) statement regarding problematic generic delimitations among these plants. Generic boundaries are difficult at best and are mostly based on differences in floral morphology that reflect adaptation to pollinators (e.g., bees and flies in *Carlowrightia* and *Henrya*; hummingbirds in *Anisacanthus*). Among genera in this lineage, only *Henrya* and *Chalarothyrsus* have unambiguous apomorphies (i.e., fused bracteoles and pollen traits in the former, seeds fused to the capsule valves in the latter) that are not likely related to recent selection by pollinators.

Interestingly, this *Tetramerium* lineage is almost exclusively New World in geographic distribution. Mexico is especially rich in its members and several genera are endemic there [i.e., *Chalarothyrsus, Gypsacanthus, Hoverdenia, Mirandea, Aphanosperma* T. F. Daniel; (the last is not included in our sample but undoubtedly belongs here on the basis of macromorphological, palynological, and cytological similarities; Daniel, 1988, 1990)] and others have their greatest concentration of species in Mexico (e.g., *Anisacanthus, Carlowrightia, Henrya, Tetramerium*). *Ecbolium* is the only Old World member in the present sample, but its placement here is not surprising: Vollesen (as cited in Balkwill & Balkwill, 1998) and Daniel (1998b) have suggested that these plants are congeneric with the North American *Yeatesia* Small, a genus not included in our analysis but which clearly belongs in this lineage. The Old World genera *Megalochlamys* Lindau, *Angkalanthus* Balf. f., and *Calycacanthus* K. Schum. likely also belong here and should be included in future work (the same is true of New World *Yeatesia* and *Aphanosperma*, as noted above). Assuming that we are correct about placement of these genera in the *Tetramerium* lineage, the group includes about 150 species.

"*Justicioids*" *and Diclipterinae lineage.* Monophyly of the "justicioids" and Diclipterinae lineage is strongly supported in the combined analysis (recall that "justicioids" is used here to include *Justicia* and allied genera, both Old and New World in distribution). Among these plants, there is a marked tendency for increased complexity in anther morphology and ornamentation compared to other Justicieae. Thus, the thecae are usually inserted at different heights on the filament and not perfectly parallel; basal appendages of various shapes and sizes occur on the thecae in many species. This lineage is also marked by evolution of a rugula: a channel-like structure on the internal surface of the upper lip formed by parallel ridges of corolla tissue. During anthesis, the style lies in this rugula, sometimes fitting so snugly that force is required to dislodge it. If we are correct in placing the evolution of the rugula here, then this structure is lost in Diclipterinae (as here delimited) above *Rhinacanthus* (see below). The genera not included in Diclipterinae are placed either as part of a nonmonophyletic grade that includes Old World "justicioids" or as part of a clade, the New World "justicioids" lineage. As will be clear from the following discussion, our analysis confirms that generic delimitations are problematic among "justicioids," adding the additional complication that neither "justicioids" as a whole nor the genus *Justicia* are monophyletic.

Old World "justicioids." The taxa represented in our analysis are not monophyletic, nor do the species of Old World *Justicia* that we have included form a monophyletic lineage. However, it is interesting that all other Old World "justicioids" are basal to the six Old World species of *Justicia* that are included. The sister-group relationship between *Rungia* (Paleotropics, ca. 20 species) and *Metarungia* (Africa, 3 species) is strongly supported in our analysis. These plants share a placenta that rises elastically from the base of the capsule at maturity (this trait is also found in *Dicliptera*, see below). In plants of both genera, the bracts have a distinctive hyaline or colored border. There is strong support for monophyly of the lineage above *Rungia* + *Metarungia*, but we know of no non-molecular evidence for this relationship. Relationships among other Old World "justicioids" are not strongly supported by our analysis, although it is interesting that the three Malagasy *Justicia* are moderately well supported as monophyletic (BS = 68, DI = 4).

Conclusions regarding relationships among Old World "justicioids" are unwarranted based on our limited sample (10 of at least 300 species) and the inconclusive pattern of relationships among these. Further, all *Justicia* species from the Old World are monophyletic in trees that are only four steps (0.2%) longer than the MP trees. Similarly, constraining all Old World "justicioids" to monophyly requires only eight additional steps (0.3%) compared to the shortest trees. On the other hand, evidence from indels suggests that the present hypothesis is correct in placing some Old World "justicioids" closer to the Diclipterinae + New World "justicioid" lineage than to other Old World

"justicioids." The three species of Malagasy *Justicia* and *J. betonica* share a 3 bp indel in the cp locus with the Diclipterinae + New World "justicioids" lineage, and these plus *J. extensa* share a 7 bp indel, also in the cp locus, with the Diclipterinae + New World "justicioids" lineage.

Students of Acanthaceae have differing opinions regarding the validity of a number of genera that are clearly closely related to Old World *Justicia* (e.g., *Monechma* Hochst., *Adhatoda* Mill., *Aulojusticia* Lindau, Old World *Siphonoglossa* Oerst., *Ascotheca* Heine, *Trichocalyx* Balf.f., *Chlamydocardia* Lindau, and *Sarojusticia* Bremek.). Adding representatives of these groups will both expand our sample of Old World "justicioids" and test their validity as genera. The geographic range of Old World "justicioids" is extensive (Africa through west Asia to southeast Asia and Australia) and under-sampled here (we have included only African, Malagasy, and south Asian species). These plants present a series of intriguing biogeographic patterns that can be usefully addressed once we have a well resolved phylogeny for a denser and geographically broader sample. The phylogenetic status of *Justicia* is discussed further under "The *Justicia* Problem," below.

Strong support for monophyly of the Diclipterinae + New World "justicioid" lineage is perhaps the most surprising component of our results. To our knowledge, this relationship has not previously been suggested, and we cannot identify any clear non-molecular synapomorphies to corroborate the strong support from molecular sequence data. Certainly this hypothesis of relationships should be tested with additional data.

Diclipterinae. There is strong support from our analysis for a monophyletic Diclipterinae, including *Rhinacanthus*. This lineage conforms to the core of Lindau's (1895) Diclipterinae in which he placed *Peristrophe*, *Hypoestes*, and *Periestes* (= *Hypoestes*), in addition to *Dicliptera*. However, Lindau included also *Tetramerium* and *Rungia* here; we have shown that these genera have relationships elsewhere in Justicieae. Further, he placed *Rhinacanthus* in Odontoneminae. In fact, *Rhinacanthus* (Old World, ca. 20 species) shares a rugula with "justicioids" and lacks a number of the morphological synapomorphies that otherwise characterize Diclipterinae. Nonetheless, molecular data (including two unique and unreversed indels in the cp locus) strongly support *Rhinacanthus* as a member of this lineage, and cytological data corroborate this placement. Diclipterinae including *Rhinacanthus* seem to share a base chromosome complement of $x = 15$. Although chromosome numbers of $n = 15$ and $n = 30$ are not uncommon elsewhere in the family, among Justicieae $n = 15$ is known only in a few species of *Justicia*. Among *Rhinacanthus*, *Peristrophe*, and *Hypoestes*, $n = 15$ and 30 are the most commonly reported numbers. Many Old World *Dicliptera* have $n = 13$, but $n = 15$ is known from both African and Malagasy species (Kaur, 1970; Daniel, unpublished data). We thus suggest that $n = 13$ represents dysploid evolution from $n = 15$. Interestingly, all New World species of *Dicliptera* for which counts have been obtained have $n = 40$ and thus appear to be ancient polyploids (Daniel, 2000; Daniel & Chuang, 1993).

Diclipterinae above *Rhinacanthus* are one of the most strongly supported lineages in our analysis; these plants are also well marked by morphological synapomorphies. Corollas of these plants lack the rugula that is otherwise characteristic of the "justicioids" and Diclipterinae lineage. *Hypoestes*, *Peristrophe*, and *Dicliptera* all share a specialized type of compound inflorescence (Balkwill & Getliffe Norris, 1988). Additionally, in these three genera the corolla is resupinate through 180 degrees of rotation. This trait has apparently been lost secondarily in a number of New World *Dicliptera*. Interestingly, in some species of New World *Dicliptera*, the corolla is resupinate through a full 360 degrees: in these plants, the corolla appears to be normally oriented, but this is not achieved in the normal way (Daniel, 1995c).

The two species of *Hypoestes* are strongly supported as each other's closest relative, and cp data place *Peristrophe* sister to these two, again with strong support (Fig. 3; these taxa also share an 11 bp indel in the cp locus). *Hypoestes* (Old World, ca. 150 species) differs from *Peristrophe* (Old World, ca. 25 species) and *Dicliptera* (Old and New World, ca. 80 species) by having monothecous anthers. We know of no non-molecular synapomorphies that mark the *Hypoestes* + *Peristrophe* clade.

Chloroplast data (including three indels, one ca. 50 bp long) place all five species of *Dicliptera* together, and the combined data set provides strong support for monophyly of the three species (including two from the New World and one from the Old World) for which we have sequence data for both genic regions. Species of *Dicliptera* have fruits with elastic dehiscence of the placentae; this distinguishes them from other Justicieae except *Rungia* + *Metarungia*, which also have this trait, as noted above. Our results agree with the conclusions of Balkwill and Getliffe Norris (1988) in indicating that elastic placentae evolved separately in these two groups; subtle differences in the trait are to be sought. In fact, fruits of a number of other genera

including *Tetramerium* and *Henrya* in Justicieae have placentae that separate from the fruit wall, but are not considered elastic (Daniel, 1986, 1990). These characters merit further comparative study across Acanthaceae to identify homologies and analogies.

Diclipterinae, with an estimated diversity of 300 species, have not suffered from the proliferation of small genera observed in other lineages of Justicieae. *Dicliptera* and *Hypoestes* are large genera whose monophyly should be tested. Especially interesting is that the biogeographic range of *Dicliptera* has a cytological correlate, as noted above; phylogenetic work within this genus is certainly warranted.

New World "Justicioid" lineage. Our results suggest the existence of a monophyletic group that includes all New World *Justicia* and members of related genera. In addition to sequence support for this relationship, all taxa share two indels in the cp locus. Generic limits among New World "justicioids" have been unsettled for some time. For example, among species included here, *J. brandegeana* was described originally in *Beloperone* and subsequently moved to its own genus, *Calliaspidia* Bremek., by Bremekamp (1948). *Justicia longii* was treated in *Siphonoglossa* until this group was studied by Hilsenbeck (1990). Our analysis indicates that submerging these genera in *Justicia* was warranted. In her infrageneric classification of *Justicia*, Graham (1988) included these and many other genera in *Justicia*. Daniel (1991) noted that *Poikilacanthus* (ca. 14 species) has but little to distinguish it from New World *Justicia* except pollen morphology, as was also noted by Raj (1961) and Bremekamp (1965). In fact, among species of New World *Justicia* there is already a remarkably rich diversity of pollen morphology (Daniel, 1998a). *Megaskepasma*, with a single Neotropical species, is a plant with spectacular magenta bracts, long white corollas, and pollen that is similar to at least one species of *Poikilacanthus* (Daniel, 1991, 1998a). Finally, *Harpochilus* (3 species) is a poorly known genus endemic to Brazil with highly specialized corollas. Morphological distinctions between this genus and *Justicia* have not been fully investigated.

We estimate that the New World "justicioid" lineage includes about 400 species, with much still remaining to be discovered about species-level diversity. In this context, our sample of eight species (including the three for which we have only nr ITS data) is extremely sparse. On the other hand, our sample is taxonomically diverse, as described above, such that we regard the hypothesis that the entire group is monophyletic as robust. However, species representing other genera, both currently recognized as well as those already synonymized with *Justicia*, should be included (e.g., *Sebastiano-Schaueria* Nees, *Clistax* Mart., *Chaetochlamys* Lindau, *Chaetothylax* Nees, *Neohallia* Hemsl., *Tabascina* Baill.). On the other hand, as indicated by branch lengths in Figure 4, there is remarkably little molecular diversity among the sampled species. For both the nr ITS and *trn*L-*trn*F sequences, New World "justicioids" have lower rates of divergence than their sister group (Diclipterinae). These low levels of molecular divergence contrast with species diversity (the New World "justicioid" lineage is as species-rich as any other lineage of Justicieae), as well as with morphological and cytological disparity. Plants of this lineage range from prostrate herbs to trees, have corollas from a few mm to at least 8.5 cm in length, and have a startling range of diversity in pollen morphology (Daniel, 1998a). At least 11 different chromosome numbers, ranging from $n = 11$ to $n = 31$, have been reported for New World species of *Justicia* alone. It is remarkable that this explosion of morphological and cytological diversity is not reflected by molecular divergence, at least at these loci.

The Justicia *problem.* Our results indicate that *Justicia* is not monophyletic, that New World *Justicia* is monophyletic only if a number of other "justicioids" are included, and that Old World species of *Justicia* are unlikely to form a monophyletic group whether or not other "justicioids" are included. Clearly, a great deal remains to be learned about phylogenetic relationships of plants described in this genus. Even species-level diversity remains poorly documented among "justicioids": *Index Kewensis* (Davies, 1991, 1996) reports 81 new species described in *Justicia* alone between 1986 and 1995.

The results of our analysis point to a strong phylogenetic distinction between Old and New World "justicioids," despite the fact that they share many macromorphological characteristics, have similar chromosome number patterns, and the same wide range of pollen types. Constraining New + Old World "justicioids" to monophyly requires 32 additional steps (1.5%) compared to the shortest trees. There are no established benchmarks against which to evaluate an increase of 1.5% in tree length, but note that decay indices (Figs. 2, 3) indicate that only Diclipterinae above *Rhinacanthus* would be resolved in trees 30 steps longer than the MP trees. That is, trees in which all "justicioids" are monophyletic would lack resolution among essentially all other Justicieae.

In her infrageneric classification of *Justicia*

based on study of 295 species of the genus, Graham (1988) recognized 16 sections. Interestingly, Graham's work seems to have presaged our results in that none of her sections have species in both hemispheres (7 are restricted to the Old World, 9 to the New World). However, the characters used to distinguish the sections often seem extremely fine [e.g., the distinctions between the Old World sect. *Justicia* and the New World sect. *Drejerella* are based on corolla color (cream versus red) and size (less than 25 mm versus more than 35 mm)]. We have thus tended to view her work as a monumental step toward characterizing variation among species of *Justicia,* but still preliminary. Our sample of "justicioids" does not permit evaluation of Graham's (1988) sections, but she seems to have been correct in separating New and Old World members. Clearly, our own contribution can be viewed as preliminary as well in that we are still far from a full understanding of relationships among all "justicioids."

Conclusions

The morphological diversity among Justicieae as well as the sheer size of the group in terms of number of species has made coming to terms with infratribal relationships quite challenging. In such diverse groups, the simplicity of molecular sequence data simplifies comparison. More importantly, placing comparative data in a phylogenetic context permits distinguishing shared derived characters that argue for relationships from shared primitive characters that do not.

It will be clear from the foregoing discussion that Lindau (1895) correctly diagnosed Diclipterinae and Isoglossinae (although he placed in both of these subtribes some genera that we demonstrate belong elsewhere) but otherwise shuffled things up rather thoroughly. Bremekamp (1965) was likewise correct in recognizing Isoglossinae (his Rhytiglossinae) and Justiciinae. The fact that he did not include Diclipterinae in the latter is mirrored by our inability to point to non-molecular synapomorphies that support the embedding of Diclipterinae in Bremekamp's Justiciinae (our "justicioids"). Bremekamp's Odontoneminae thus included Diclipterinae (except *Rhinacanthus*), the *Tetramerium* lineage, and the *Pseuderanthemum* lineage, a non-monophyletic group of plants whose morphological basis lies in retention of primitive characters. More recently, Balkwill and Getliffe Norris (1988) presented a classification for southern African Acanthaceae that improves upon Bremekamp's classification by removing Diclipterinae (again, as here defined except that *Rhinacanthus* is placed with their Justiciinae) from Odontoneminae. These authors also recognize Isoglossinae, Justiciinae (our Old World "justicioids" plus *Rhinacanthus*), and Odontoneminae. This latter group includes elements of our *Pseuderanthemum* and *Tetramerium* lineages. Finally, our results corroborate the findings of McDade and Moody (1999) and McDade et al. (2000) regarding clades within Justicieae, while expanding the sample of Justicieae considerably. In particular, these earlier analyses included no Old World "justicioids."

As discussed throughout, our results are limited in a number of ways. First, although much of our phylogenetic hypothesis is remarkably strongly supported, a few key aspects are not. Notably, establishing the phylogenetic status of the *Pseuderanthemum* lineage with confidence requires additional research effort. Second, in some lineages (notably most of the *Pseuderanthemum* lineage and New World "justicioid" lineage), the genic regions examined here do not provide sufficient variation to elucidate relationships with confidence. It will be necessary to add another, more rapidly evolving locus to our molecular tools in order to unravel relationships in these groups and at lower taxonomic levels than explored here. Third, sampling remains insufficiently dense to address all of the interesting phylogenetic problems in Justicieae, notably with regard to Old World "justicioids." Stating this shortcoming more positively, our results provide a framework to which additional taxa can be readily added to address specific questions regarding phylogenetic relationships. In addition to taxa whose characters permit predictions about their placement into this framework, a few present character combinations that defy predictions (e.g., *Leandriella* Benoist with biporate pollen similar to "core" Isoglossinae and four stamens characteristic of basal Justicieae). Fourth, in many cases, our knowledge of morphology, palynology, and cytology among Justicieae remains inadequate for the sort of large-scale, comparative project that we have undertaken. We anticipate addressing some of the limitations of the present study in continuing collaborative efforts.

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Appendix 1.

Taxa, Genbank accession numbers (nr ITS followed by *trn*L-*trn*F; NA = not available), and sources of plant materials from which DNA was extracted for sequencing of the nuclear ribosomal internal transcribed spacer region and chloroplast *trn*L-*trn*F spacer and intron. Abbreviations for herbaria follow Holmgren et al. (1990). Fresh material or material placed directly into silica gel was used except as indicated (HS = herbarium specimen). When plants in cultivation were used, we provide also information on native range. Classification follows results presented herein with regard to Justicieae (with taxa listed alphabetically within lineage), and Manktelow et al. (in press) with regard to others. To facilitate phylogenetic placement of taxa of interest, Appendix 2 lists Justicieae alphabetically and assigns each to a lineage (or grade in the case of Old World "justicioids").

Taxon; Genbank accession number (nr ITS, *trn*L-*trn*F); source and voucher information.

Acanthoideae

Stenandrium pilosulum (S. F. Blake) T. F. Daniel; AF169758, AF061827; Mexico. Sonora: Yécora Municipio, El Kipor, *Van Devender & Reina G. 97-434* (ARIZ).

Aphelandra campanensis Durkee; AF169760, AF061829; Panama. San Blas: near Mandinga, Río Mandinga, *McDade 852* (DUKE).

Ruellioideae

Ruellieae s.l.

Sanchezia speciosa Leonard; AF169385, AF063113; Cultivated, Duke University greenhouses, Durham, North Carolina, U.S.A., Accession No. 66-462 (native to South America), *McDade 1180* (ARIZ).

Ruellia californica (Rose) I. M. Johnst.; AF167704, AF063115; Cultivated, Univ. Arizona campus, Tucson, Arizona, U.S.A., *McDade 1157* (ARIZ) (native to northwest Mexico).

Barlerieae

Barleria lupulina Lindl.; AF169751, AF289758; Cultivated, San Francisco Conservatory of Flowers, San Francisco, California, U.S.A., *Daniel s.n.* (CAS) (native to Mauritius).

Lepidagathis villosa Hedrén; AF169752, AF063121; Scotland et al. (1995) [DNA provided by R. Olmstead (University of Washington) and R. Scotland (Oxford University)].

Justicieae

Pseuderanthemum Lineage

Asystasia gangetica (L.) T. Anderson; AF289793, AF289748; Cultivated, Nelspruit, Mpumalanga, South Africa, *Daniel & Balkwill 9386* (CAS) (widespread in Old World tropics and subtropics).

A. sp.; AF289794, AF289749; Madagascar. Fianarantsoa, Ranomafana National Park, *Daniel 9129* (CAS).

Chileranthemum pyramidatum (Lindau) T. F. Daniel; AF289797, AF289752; (HS) Mexico. Chiapas: Municipio La Trinitaria, *Breedlove & Daniel 70767* (CAS).

Daniel 6737cv; AF289799, AF289754; (HS) Cultivated, San Francisco Conservatory of Flowers, San Francisco, California, U.S.A., *Daniel 6737cv* (CAS) (native to Madagascar).

Herpetacanthus stenophyllus Gómez-Laur. & Grayum; AF289795, AF289750; (HS) Costa Rica. Limón: Reserva Biología Hitoy Cerere, *Herrera 3855* (ARIZ).

Mackaya bella Harv.; AF289796, AF289751; Cultivated, Strybing Arboretum, San Francisco, California, U.S.A., *Daniel s.n.* (CAS) (native to southern Africa).

Odontonema tubaeforme (Bertol.) Kuntze; AF169748, AF063127; Cultivated, Duke University greenhouses, Durham, North Carolina, U.S.A., Accession No.

66-153, *McDade 1182* (ARIZ) (native to Mexico and Central America).

Oplonia microphylla (Lam.) Stearn; AF289798, AF289753; (HS) Cultivated, San Francisco Conservatory of Flowers, San Franciso, California, U.S.A., *Ornduff 7814cv* (CAS) (native to the West Indies).

Pseuderanthemum alatum (Nees) Radlk.; AF169749, AF063130; Cultivated, Duke University greenhouses, Durham, North Carolina, U.S.A., Accession No. 84-055, *McDade 1183* (ARIZ) (native to North and Central America).

Ruspolia seticalyx (C. B. Clarke) Milne-Redh.; AF289800, AF289755; (HS) Cultivated, Waimea Arboretum and Botanical Garden, Oahu, Hawaii, U.S.A., *Daniel & Butterwick 6635* (CAS) (native to Africa).

Ruttya fruticosa Lindau; AF289801, AF289756; Cultivated, San Francisco Conservatory of Flowers, San Francisco, California, U.S.A., *Daniel s.n.* (CAS) (native to Africa).

Spathacanthus hoffmannii Lindau; AF289802, NA; (HS) Costa Rica. San José: Reserva Biológica Carara, *Morales 1347* (ARIZ).

S. parviflorus Leonard; AF289803, AF289757; (HS) Mexico. Chiapas: El Triunfo Biosphere Reserve, *Daniel et al. 8403* (CAS).

Isoglossinae

Brachystephanus lyallii Nees; AF289790, NA; Madagascar. Fianarantsoa, Ranomafana National Park, *Daniel 9101* (CAS).

Isoglossa grandiflora C. B. Clarke; AF289788, AF289745; (HS) Cultivated, San Francisco Conservatory of Flowers, San Francisco, California, U.S.A., *Daniel s.n.* (CAS) (native to eastern tropical Africa).

Isoglossa? sp. *9106*; AF289789, AF289746; Madagascar. Fianarantsoa, Ranomafana National Park, *Daniel 9106* (CAS).

Ptyssiglottis pubisepala (Lindau) B. Hansen; AF289787, AF289744; (HS) Papua New Guinea. Madang, ca. 8 km SSW of Madang, *Daniel 6630* (CAS).

Razisea spicata Oerst.; AF169848, AF063131; Costa Rica. Heredia: La Selva Biological Station, *Hammel 7974* (DUKE).

Stenostephanus chiapensis T. F. Daniel; AF289792, AF289747; Cultivated, San Francisco, California, U.S.A., *Breedlove & Burns 72688cv* (CAS) (native to southern Mexico).

S. silvaticus (Nees) T. F. Daniel; AF169747, AF063132; (HS) Costa Rica. San José: Parque Nacional Braulio Carrillo, *Maas 7800* (MO).

Tetramerium Lineage

Anisacanthus puberulus (Torr.) Henr. & E. J. Lott; AF289778, NA; Cultivated, Univ. Arizona campus, Tucson, Arizona, U.S.A., *McDade 1179* (ARIZ) (native to southwestern U.S.A. and northern Mexico).

A. thurberi (Torr.) A. Gray; AF169846, AF063122; U.S.A. Arizona: Pima County, Tucson Mountains, *Van Devender 88-150* (ARIZ).

Carlowrightia arizonica A. Gray; AF169845, AF063123; U.S.A. Arizona: Pima County, Tucson Mountains, *Jenkins 89-24* (ARIZ).

Chalarothyrsus amplexicaulis Lindau; AF289780, AF289740; (HS) Cultivated, San Francisco Observatory of Flowers, California, U.S.A., *Daniel & Bartholomew 4842gh* (CAS) (native to western Mexico).

Ecbolium syringifolium (Vahl) Vollesen; AF289786, AF289743; (HS) Madagascar. Toliara, Taolanaro, *Daniel & Butterwick 6733* (CAS).

Fittonia albivenis (Lindl. ex Veitch) Brummitt; AF289781, AF289741; Cultivated, Univ. Arizona, Tucson, Arizona, U.S.A., *McDade 1178* (ARIZ) (native to South America).

Gypsacanthus nelsonii E. J. Lott, V. Jaram. & Rzed.; AF289779, AF289739; Mexico. Puebla: Municipio Coxcatlán, *Daniel 8357* (CAS).

Henrya insularis Nees ex Benth.; AF169843, AF063125; Mexico. Sonora: near Alamos, *Jenkins 89-432* (ARIZ).

Hoverdenia speciosa Nees; AF289777, AF289738; (HS) Mexico. Hidalgo: Barranca de Tolantongo, *Daniel & Baker 3739* (CAS).

Mirandea grisea Rzed.; AF289783, NA; (HS) Mexico. San Luis Potosí: ca. 3 km NW of La Calzada, *Daniel & Baker 3717* (CAS).

Pachystachys lutea Nees; AF169844, AF063128; Cultivated, Duke University greenhouses, Durham, North Carolina, U.S.A., Accession No. 84-055 (native to Peru), *McDade 1181* (ARIZ).

Schaueria calicotricha (Link & Otto) Nees; AF289782, NA; (HS) Cultivated, Homestead, Florida, U.S.A., *Foote s.n.* (CAS) (native to Brazil).

Streblacanthus cordatus Lindau; AF289784, AF289742; Panama. Colón: vicinity of Portobelo, *Daniel et al. 8203* (CAS).

S. roseus (Radlk.) B. L. Burtt; AF289785, NA; Cultivated, San Francisco Conservatory of Flowers, San Francisco, California, U.S.A., *Daniel s.n.* (CAS) (native to Peru).

Tetramerium nervosum Nees; AF169847, AF063133; U.S.A. Arizona: Pima County, near Patagonia, *McDade & Jenkins 1154* (ARIZ).

Old World "justicioids" (note that the results reported herein indicate that this is not a monophyletic group but rather a grade).

Anisotes madagascariensis Benoist; AF289772, AF289733; (HS) Madagascar. Toliara: ca. 20 km N of Toliara, *Daniel & Butterwick 6736* (CAS).

Duvernoia aconitiflora A. Meeuse; AF289774, AF289735; Cultivated, Roodepoort, Witwatersrand National Botanic Garden, Gauteng, South Africa, *Daniel et al. 9361* (CAS) (native to southern Africa).

Justicia adhatoda L.; AF289773, AF289734; Cultivated, Univ. Arizona campus, Tucson, Arizona, U.S.A., *Barr 60-393* (ARIZ) (native to southern Asia).

J. betonica L.; AF289770, AF289731; Cultivated, Johannesberg, Gauteng, South Africa, *Daniel 9369* (CAS) (native to eastern and southern Africa and the Indian Subcontinent).

J. extensa T. Anderson; AF289771, AF289732; Cultivated, San Francisco Conservatory of Flowers, San Francisco, California, U.S.A. *Daniel s.n.* (CAS) (native to eastern tropical Africa).

J. sp. *9024*, AF289768, AF289729; Madagascar. Fianarantsoa, Ranomafana National Park, *Daniel 9024* (CAS).

J. sp. *9010*; AF289769, AF289730; Madagascar. Fianarantsoa, Ranomafana National Park, *Daniel 9010* (CAS).

J. sp. *983*; AF289767, AF289728; Madagascar. Masoala Peninsula, Vokoanina Forest, *Zjhra 983* (CAS).

Metarungia galpinii (C. Baden) C. Baden; AF289776, AF289737; Cultivated, Johannesberg, Gauteng, South Africa, *Daniel 9322* (CAS) (native to South Africa).

Rungia klossii S. Moore; AF289775, AF289736; (HS)

Papua New Guinea. Madang, Bundi, *Daniel et al. 6561* (CAS).

Diclipterinae

Dicliptera extenta S. Moore, NA, AF289724; Cultivated, Johannesberg, Gauteng, South Africa, *McDade 1306* (J) (native to southern Africa).

D. magaliesbergensis K. Balkwill, NA, AF289725; Witwatersrand National Botanic Garden, Gauteng, South Africa, *Daniel et al. 9357* (CAS).

D. resupinata (Vahl) Juss.; AF169841, AF063124; U.S.A. Arizona: Pima County, Santa Catalina Mountains, *Van Devender 84-269* (ARIZ).

D. suberecta (André) Bremek.; AF289763, AF289722; Cultivated, Tucson Botanical Garden, Tucson, Arizona, U.S.A., *McDade 1176* (ARIZ) (native to Uruguay).

D. sp. *9194*; AF289764, AF289723; Madagascar. Fianarantsoa, Ranomafana National Park, *Daniel 9194* (CAS).

Hypoestes aristata R. Br.; AF289765, AF289726; (HS) Cultivated, Mildred E. Mathias Botanical Garden, Los Angeles, California, U.S.A., *Daniel s.n.* (CAS) (native to tropical and southern Africa).

Hypoestes phyllostachya Baker; AF169842, AF167703; Cultivated, Univ. Arizona, Tucson, Arizona, U.S.A., *McDade 1232* (ARIZ) (native to Madagascar).

Peristrophe hyssopifolia (Burm. f.) Bremek.; NA, AF063129; Cultivated, Missouri Botanical Garden, St. Louis, Missouri, U.S.A., Accession No. MBG861410, *MacDougal 5047* (MO) (native to India).

Rhinacanthus gracilis Klotzsch; AF289766, AF289727; Cultivated, San Francisco Conservatory of Flowers, San Francisco, California, U.S.A., *Daniel s.n.* (CAS) (native to Africa).

New World Justicioids

Harpochilus neesianus Mart. ex. Nees; AF289762, AF289721; (HS) Brazil. Bahia: Santa Luzia, *Souza et al. 5413* (CAS).

Justicia brandegeana Wassh. & L. B. Sm.; AF289759, NA; Cultivated, Univ. Arizona campus, Tucson, Arizona, U.S.A., *Starr c.32* (ARIZ) (native to Mexico).

J. caudata A. Gray; AF169837, AF063134; Mexico. Sonora: near Alamos, *Faivre 64* (ARIZ).

J. comata (L.) Lam.; AF289760, NA; Costa Rica. Heredia: La Selva Biological Station, *Faivre 59* (ARIZ).

J. longii Hilsenb.; AF169839, AF063135; U.S.A. Arizona: Pima County, Tucson Mountains, *Van Devender 87-307* (ARIZ).

J. spicigera Schltdl.; AF289761, NA; Cultivated, Univ. Arizona campus, Tucson, Arizona, U.S.A., *McDade 1158* (ARIZ) (native to Mexico and Central America).

Megaskepasma erythrochlamys Lindau; AF169840, AF063126; Cultivated, Wilson Botanical Garden, Puntarenas, Costa Rica, *McDade 253* (DUKE) (native to northern South America).

Poikilacanthus macranthus Lindau; AF169838, AF07066; (HS) Costa Rica. Alajuela Province: Monteverde Reserve, *Haber 707* (MO).

Appendix 2. Alphabetical list of Justicieae included in the present analysis; each taxon is assigned to a lineage (or grade in the case of Old World "justicioids"), reflecting the results presented herein. See Appendix 1 for authors of names, Genbank accession numbers, and vouchers.

Aniscanthus puberulus	*Tetramerium* lineage
A. thurberi	*Tetramerium* lineage
Anisotes madagascariensis	Old World "justicioids"
Asystasia gangetica	*Pseuderanthemum* lineage
Asystasia sp. (*Daniel 9129*)	*Pseuderanthemum* lineage
Brachystephanus lyallii	Isoglossinae
Carlowrightia arizonica	*Tetramerium* lineage
Chalarothyrsus amplexicaulis	*Tetramerium* lineage
Chileranthemum pyramidatum	*Pseuderanthemum* lineage
Daniel 6737cv (Unidentified)	*Pseuderanthemum* lineage
Dicliptera extenta	Diclipterinae
D. magaliesbergensis	Diclipterinae
D. resupinata	Diclipterinae
D. suberecta	Diclipterinae
D. sp. (*Daniel 9194*)	Diclipterinae
Duvernoia aconitiflora	Old World "justicioids"
Ecbolium syringifolium	*Tetramerium* lineage
Fittonia albivenis	*Tetramerium* lineage
Gypsacanthus nelsonii	*Tetramerium* lineage
Harpochilus neesianus	New World "justicioid" lineage
Henrya insularis	*Tetramerium* lineage
Herpetacanthus stenophyllus	*Pseuderanthemum* lineage
Hoverdenia speciosa	*Tetramerium* lineage
Hypoestes aristata	Diclipterinae
H. phyllostachya	Diclipterinae
Isoglossa grandiflora	Isoglossinae
Isoglossa? sp. (*Daniel 9106*)	Isoglossinae
Justicia adhatoda	Old World "justicioids"
J. betonica	Old World "justicioids"
J. brandegeana	New World "justicioid" lineage
J. caudata	New World "justicioid" lineage
J. comata	New World "justicioid" lineage
J. extensa	Old World "justicioids"
J. longii	New World "justicioid" lineage
J. spicigera	New World "justicioid" lineage
J. sp. (*Daniel 9024*)	Old World "justicioids"
J. sp. (*Daniel 9010*)	Old World "justicioids"
J. sp. (*Zjhra 983*)	Old World "justicioids"
Mackaya bella	*Pseuderanthemum* lineage
Megaskepasma erythrochlamys	New World "justicioid" lineage
Metarungia galpinii	Old World "justicioids"
Mirandea grisea	*Tetramerium* lineage
Odontonema tubaeforme	*Pseuderanthemum* lineage
Oplonia microphylla	*Pseuderanthemum* lineage
Pachystachys lutea	*Tetramerium* lineage
Peristrophe hyssopifolia	Diclipterinae
Poikilacanthus macranthus	New World "justicioid" lineage
Pseuderanthemum alatum	*Tetramerium* lineage
Ptysiglottis pubisepala	Isoglossinae
Razisea spicata	Isoglossinae
Rhinacanthus gracilis	Diclipterinae
Rungia klossii	Old World "justicioids"
Ruspolia seticalyx	*Pseuderanthemum* lineage
Ruttya fruticosa	*Pseuderanthemum* lineage
Schaueria calicotricha	*Tetramerium* lineage
Sparthacanthus hoffmannii	*Pseuderanthemum* lineage
S. parviflorus	*Pseuderanthemum* lineage
Stenostephanus chiapensis	Isoglossinae
S. silvaticus	Isoglossinae
Streblacanthus cordatus	*Tetramerium* lineage
S. roseus	*Tetramerium* lineage
Tetramerium nervosum	*Tetramerium* lineage
Unidentified (*Daniel 6737cv*)	*Pseuderanthemum* lineage

A PHYLOGENETIC ANALYSIS OF *DICOMA* CASS. AND RELATED GENERA (ASTERACEAE: CICHORIOIDEAE: MUTISIEAE) BASED ON MORPHOLOGICAL AND ANATOMIC CHARACTERS[1]

Santiago Ortiz[2]

Abstract

This study reports a phylogenetic analysis of the genus *Dicoma* and the related genera *Achyrothalamus, Erythrocephalum, Pasaccardoa,* and *Pleiotaxis* (Mutisieae, Asteraceae), which share a series of morphological characters of the corolla, ray floret epidermis, anther appendages, and style branches. A matrix of 77 morphological and anatomical characters was used for the analysis. The genera *Gochnatia* and *Oldenburgia* were used as outgroups. The topology of the resulting consensus cladogram suggests that the genus *Dicoma* appears paraphyletic. A clade including *Dicoma* and *Pasaccardoa* appears monophyletic with subgroups clearly defined by a large number of synapomorphies. These findings indicate that maintenance of *Pasaccardoa* as an independent genus implies a need to divide *Dicoma* into several genera. The genera *Pleiotaxis, Achyrothalamus,* and *Erythrocephalum* form a monophyletic group. Issues relating to the geographical origin of *Dicoma* (probably Madagascar or southern Africa), and to the evolution of the different species groups, are briefly discussed.

Key words: Asteraceae, cladistics, *Dicoma,* Mutisieae, phylogeny, tropical Africa.

The "*Dicoma* group" (Asteraceae, Mutisieae, Mutisinae) of Bremer (1994) is considered by this author to be one of the most difficult groups to evaluate phylogenetically within the Mutisieae. According to Bremer (1994), it is characterized by its corolla distinctly divided into a narrow tube and wide limb, its non-mutisioid ray floret epidermis pattern, its acuminate apical anther appendages, and its mostly subapically pilose style branches. It comprises *Dicoma* itself, with 50 species occurring in tropical Africa, Madagascar, and South Africa, and two species reaching Asia (*D. schimperi* (DC.) Baill. ex O. Hoffm. is present in the Arabian Peninsula, and *D. tomentosa* Cass. is present in India and Pakistan). Other genera of this group are *Erythrocephalum* Benth. with about 12 species occurring throughout tropical Africa (particularly tropical East Africa), *Achyrothalamus* O. Hoffm. with a single species from Kenya and Tanzania, *Pleiotaxis* Steetz with about 25 species occurring throughout tropical Africa, *Pasaccardoa* Kuntze with 4 species from tropical Africa, and *Gladiopappus* Humbert with a single species, possibly extinct, from Madagascar.

Prior to Bremer's (1994) "*Dicoma* group," similar groupings had been proposed by Jeffrey (1967) and Grau (1980). Indeed, Jeffrey considered this group to be one of the most distinct within the Mutisieae. Hansen (1991) suggested that most of the genera of this group be moved from the Mutisieae to the tribe Cynareae (Cardueae). His support included (a) the cuticular ornamentation of corolla epidermal cells; (b) the corolla divided into a narrow tube and a broader limb; (c) bilabiate flowers with upper limb lobules short and uncoiled or absent (except in *Gladiopappus,* in which they are long and coiled; Humbert, 1963); and (d) style branches with subapical sweeping hairs. Karis et al.'s (1992) cladistic analysis of the subfamily Cichorioideae indicated that *Dicoma, Erythrocephalum,* and *Pleiotaxis* form a monophyletic group within the tribe Mutisieae, in accordance with traditional views (see Hoffmann, 1893a; Cabrera, 1977).

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Table 1. Section assignments of the *Dicoma* species included in the present study, following the proposals of Hoffmann (1893a) and Wilson (1923). Question marks indicate that the species in question has not been assigned previously to any section, either by Hoffmann or Wilson or by subsequent authors; for these species, the assignment shown is mine, on the basis of morphological characters.

Hoffmann (1893a)				Wilson (1923)		
Steirocoma DC.	*Rhigiothamnus* (Less.) DC.	*Eudicoma* DC.	*Macledium* (Cass.) DC.	*Dimorphae* Wilson	*Barbellatae* Wilson	*Plumosae* Wilson
D. capensis	*D. picta*	*D. tomentosa*	*D. spinosa*	*D. tomentosa*	*D. anomala*	*D. sessiliflora*
D. niccolifera?	*D. fruticosa?*	*D. bangueolensis?*	*D. relhanioides*	*D. bangueolensis*	*D. elegans*	*D. zeyheri*
D. elegans?				*D. capensis*	*D. picta*	*D. saligna?*
				D. welwitschii	*D. nachtigalii*	
Hochstetteria (DC.) O. Hoffm.	*Psilocoma* Harv.	*Brachyachaenium* Baker	*Pterocoma* DC.		*D. spinosa*	
D. schimperi	*D. anomala*	*D. nachtigalii*	*D. sessiliflora*		*D. relhanioides*	
	D. cana		*D. zeyheri*		*D. dinteri*	
	D. dinteri?		*D. saligna?*		*D. cana*	
	D. aethiopica?				*D. niccolifera?*	
	D. grandidieri?				*D. fruticosa?*	
	D. montana?				*D. aethiopica?*	
	D. gillettii?				*D. grandidieri?*	
	D. carbonaria?				*D. montana?*	
	D. oleaefolia?				*D. gillettii?*	
	D. welwitschii?				*D. carbonaria?*	
					D. oleaefolia?	
					D. schimperi?	

Table 2. List of the species included in the study, showing voucher specimens and distribution.

Species	Voucher specimens	Distribution
Achyrothalamus		
A. marginatus O. Hoffm.	*Faulkner 3980* (K), *Pocs 88056* (K)	Kenya, Tanzania
Dicoma Cass.		
D. aethiopica S. Ortiz & Rodr. Oubiña	*Gilbert, Ensermu & Vollesen 7870* (K)	Ethiopia
D. anomala Sond.	*M. da Silva 3181* (COI), *Gossweiler 4143* (COI)	Tropical Africa and southern Africa
D. bangueolensis Buscal. & Muschl.	*Beckett 242* (K)	Somalia
D. cana Balf f.	*Balfour 157* (K)	Socotra
D. capensis Less	*Bryant 104* (K), *Schlieben 8938* (BM)	Southern Africa
D. carbonaria (S. Moore) Humbert	*Chauvet 359* (K), *Decary 9263* (MO)	Madagascar
D. dinteri S. Moore	*Dinter 3525* (K), *Merxmüller 881* (BM)	Namibia
D. elegans Welw. ex O. Hoffm.	*Antunes 71* (COI), *Gossweiler 1822* (COI)	Angola
D. fruticosa Compton	*Van Breda* 4426 (K)	South Africa (Cape Province)
D. gillettii Rodr. Oubiña & S. Ortiz	*Gillett & Beckett 23263* (K)	Somalia
D. grandidieri (Drake) Humbert	*Capuron 18643-SF* (K), *Decary 10707* (BM)	Madagascar
D. montana Schweick.	*L.E. Codd 8689* (K), *Dyer 9169* (BM)	South Africa (Transvaal)
D. nachtigalii O. Hoffm.	*Pearson 7642* (K)	Angola, Namibia
D. niccolifera Wild	*Walters 2347* (K), *Leach 8928* (K)	Zambia, Zimbabwe
D. oleaefolia Humbert	*Kaudern s.n.* (K)	Madagascar
D. picta (Thunb.) Druce	*Acocks 19004* (K)	South Africa (Cape Province)
D. relhanioides Less.	*Acocks 5578* (S)	South Africa (Cape Province)
D. saligna Lawalrée	*Gossweiler 11331* (COI), *Exell & Mendonça 3032* (COI)	Zambia, Angola, Congo
D. schimperi (DC.) Baill. ex O. Hoffm.	*Bally & Melville 15857* (K), *Thesiger s.n.* (BM)	Arabian Peninsula, Djibouti, Egypt, Sudan, Somalia
D. sessiliflora Harv.	*Wingfield 4416* (K), *Schleiben 5913* (BM)	Tropical Africa
D. spinosa (L.) Druce	*Foucarde 2499* (K), *Schlechter 9794* (BM)	South Africa (Cape Province)
D. tomentosa Cass.	*M. da Silva 2923* (COI), *Nordenstan 2454* (S)	Pakistan, India, tropical Africa, southern Africa
D. welwitschii O. Hoffm.	*Exell & Mendonça 3069* (COI), *M. da Silva 3668* (COI)	Congo, Angola
D. zeyheri Sond.	*Mogg 16306* (K), *Willms 661a* (BM)	Mozambique, Swaziland, South Africa (Transvaal, Natal)
Erythrocephalum Benth.		
E. microcephalum Dandy	*Burtt 2699* (K), *Dummer 5015* (K)	Uganda, Kenya, Tanzania
E. scabrifolium C. Jeffrey	*H.M. Richards 4836* (K), *Bullock 2344* (K)	Tanzania, Congo, Zambia, Malawi, Angola
E. zambesianum Oliv. & Hiern	*Burtt 3289* (K), *Davies 367* (K)	Tanzania, Congo, Zambia, Zimbabwe, Malawi, Mozambique

Table 2. Continued.

Species	Voucher specimens	Distribution
Pasaccardoa Kuntze		
P. baumii O. Hoffm.	*Monteiro & Murta 2024* (COI), *Gossweiler 3017* (COI)	Angola
P. grantii (Benth. & Oliv.) Kuntze	*Polhill 11608* (S)	Tanzania, Congo, Zambia
P. jeffreyi Wild	*Exell & Mendonça 380* (COI), *Exell & Mendonça 365* (COI)	Congo, Zambia, Angola
Pleiotaxis Steetz		
P. pulcherrima Steetz	*Cardoso 87* (COI), *Barbosa 11577* (COI)	Tanzania, Rwanda, Burundi, Congo, Zambia, Malawi, Angola, Mozambique
P. rugosa O. Hoffm.	*F. Moreno 146* (COI), *Gossweiler 1825* (COI)	Congo, Angola
P. subpaniculata Chiov.	*Murta 103* (COI), *B. Teixeira 298* (COI)	Congo, Angola
Gochnatia		
G. amplexifolia (Gardner) Cabrera	*Hatschbach 27032* (S)	Brazil
G. attenuata (Britton) Jervis & Alain	*Ekman 19174*	Cuba
G. cordata Less.	*Malme 899a* (S)	Brazil, Paraguay, Uruguay and Argentina
G. microcephala (Griseb.) Jervis & Alain	*Ekman 9280* (S)	Cuba
G. picardae (Urb.) Jiménez	*Ekman 5385* (S)	Haiti
Oldenburgia		
O. grandis (Thunb.) Baill.	*Bayliss 4519* (S)	South Africa
O. papionum DC.	*Bond 1721* (S)	South Africa
O. paradoxa Less.	*Esterhuysen 30160* (S)	South Africa

Table 3. Characters and states used in phylogenetic analysis.

1. Woody (0) / herbaceous (1).
2. Plant erect (0) / spreading to prostrate (1).
3. Stem with conical pluricellular trichomes (0) / without pluricellular trichomes (1).
4. Leaf ovate to lanceolate (0) / obovate to oblanceolate (1).
5. Leaf herbaceous (0) / coriaceous (1).
6. Leaf margin not revolute (0) / conspicuously revolute (1).
7. Leaf not conduplicate (0) / conduplicate (1).
8. Stem without subtending leaves (0) / with subtending leaves (1).
9. Capitulum > 20 mm wide (0) / capitulum ≤ 20 mm wide (1).
10. Involucre campanulate (0) / obconic (1).
11. Outer phyllaries erect-patent to erect (0) / patent to reflexed (1).
12. Phyllary midrib inconspicuous (0) / conspicuous (1).
13. Phyllaries without longitudinal dark stripes (0) / with dark stripes running along the phyllaries (1).
14. Phyllary sclerenchymal fibers in a ± continuous tissue (0) / in distinct bundles surrounded by parenchymal tissue (1) / concentrated on the abaxial face (2) / concentrated on the adaxial face (3).
15. Resin ducts in phyllaries at the abaxial face (0) / reduced or absent (1) (see Fig. 1).
16. Phyllaries acute to acuminate-pungent (0) / subacute to obtuse (1).
17. Innermost phyllaries with scarious margins (0) / ± entirely scarious (1).
18. Innermost phyllaries longer than the rest (0) / shorter than the contiguous outer series (1).
19. Phyllary glabrescent to hairy (0) / completely glabrous (1).
20. Receptacle epaleate (0) / paleate (1).
21. Florets in capitula actinomorphic (0) / marginal florets bilabiate and disc florets actinomorphic (1) / zygomorphic (transitional between actinomorphic and ligulate) (2) / marginal florets "true-ray" (Karis et al., 1992) and disc florets actinomorphic (3).
22. Marginal florets hermaphroditic (0) / neuter (1).
23. Corollas ± as long as or only slightly longer than the involucre (0) / much longer than the involucre (1).
24. Disc corolla tube gradually dilates into limb (0) / abruptly dilates into limb (1).
25. Disc corolla lobes erect to erect-patent (0) / recurved at the apex (1).
26. Disc floret epidermal cell cuticle ornamentation: mutisioid (0) / slightly transversely ondulate-striate to nearly smooth (1) / conspicuously longitudinally striate and transversely ondulate ("intestine-like") (2) / senecioid (3).
27. Disc corolla margins not sclerified (0) / sclerified (1).
28. Disc corolla without long twin glandular hairs (0) / with long twin glandular hairs (1).
29. Disc corolla without simple hairs (0) / with simple hairs (1).

Table 3. Continued.

30. Star-shaped calcium oxalate crystals in disc corolla subepidermal cells absent (0) / present (1) (see Fig. 2).
31. Vascular tissue in disc corolla bifurcate at the lobe sinuses (0) / bifurcate well below the sinuses (1) / bifurcate at the corolla base (2).
32. Marginal vascular tissue in the disc corolla broad (0) / narrow (1).
33. Marginal vascular tissue in disc corolla conspicuously submarginal (see Fig. 3-3 and 3-4 (0) / ± marginal (Fig. 3-1 and 3-2) (1).
34. Accessory vascular strands in the disc corolla lobes absent (0) / present (1).
35. Apex of the disc corolla lobes with thick-bundled veins (0) / without thick-bundled veins (1).
36. Stamen insertion in disc corolla: at the tube/throat junction (0) / near the corolla base (1).
37. Star-shaped calcium oxalate crystals in stamen filaments absent (0) / present (1).
38. Stamen filament collar inconspicuous (0) / conspicuous and not swollen (1) / conspicuous and swollen at the apical part.
39. Stamen filament collar < 0.8 mm long (0) / > 1.5 mm long (1).
40. Anther not protruding beyond the corolla (0) / protruding beyond the corolla (1).
41. Anther appendage apiculate (0) / acute to slightly acuminate (1).
42. Anther tails calcarate (0) / ecalcarate (1).
43. Anther tails long tapering (0) / with subacute to subrounded apex (1).
44. Contiguous anther tails free (0) / joined (1).
45. Anther tail apices with antrorse ramifications (0) / without ramifications (1).
46. Apex of the anther tail ramifications obtuse (0) (Fig. 5-3) / acute (1) (Figs. 5-1 and 5-2).
47. Sporoderm thick (0) / thin (1).
48. Pollen smooth to slightly granulate (0) / echinate (1).
49. Style with two veins (0) / four veins (1).
50. Star-shaped crystals in styles absent (0) / present (1).
51. Style branches adjacent (0) / style branches separate (1).
52. Style branches straight (0) / conspicuously curved (1).
53. Style branches short (0) (Figs. 6-1 and 6-2) / long (1) (Figs. 6-3 and 6-4).
54. Stylar sweeping hairs absent (0) / reaching or almost reaching the bifurcation (1) (Figs. 6-1 and 6-2) / in a long group not reaching the bifurcation (2) (Figs. 6-4) / in a subapical tuft (3) (Fig. 6-3).
55. Stylar sweeping hairs of similar length (0) (Fig. 6-1) / with a sub-basal group slightly longer than the rest (1) (Fig. 6-2) / with a sub-basal group conspicuously longer than the rest (2) (Figs. 6-3 and 6-4).
56. Style branch vascular tissue narrow (0) / very thick (1).
57. Cypsela carpopodium present (0) / absent (1).
58. Disc floret cypsela ellipsoid (0) / obovoid to obconic (1).

Table 3. Continued.

59. Cypsela of marginal florets without rostrum (0) / with rostrum (1).
60. Cypsela ± smooth (0) / conspicuously ribbed (1).
61. Star-shaped crystals in the cypsela pericarp absent (0) / present (1).
62. Superficial cypsela glands absent (0) / in a continuous layer (1) / on the ribs (2) / in the intercostal grooves (3).
63. Cypsela twin hairs simple (0) / twin hairs bifurcate (1) / absent (2).
64. Twin hairs all around the cypsela (0) (Fig. 7-2) / between ribs (1) (Fig. 7-1 and 7-3).
65. Twin-hair bases not bulbous-glandular (0) / conspicuously bulbous-glandular (1).
66. Twin hairs of the cypsela base similar to those of the other parts of the cypsela (0) / conspicuously enlarged (1).
67. Cypsela with biseriate glands (0) / without biseriate glands (1).
68. Immature testa with similar proportion of needle crystals and square to short-rectangular non-oriented crystals (0) / with long-rectangular crystals oriented in the same direction (1).
69. Mature testa pattern: *Gochnatia* type (0) / *Erythrocephalum* type (1) / *Dicoma* type (2) / *Dicoma welwitschii* type (3).
70. Testa epidermal cells with the lateral and basal walls strengthened (0) / only the basal walls strengthened (1).
71. Pappus of bristles (0) / scales (1) / bristles and scales (2) / absent (3).
72. Pappus bristles scabrid to barbellate (0) / plumose (1).
73. Pappus element persistent (0) / caducous (1).
74. Pappus elements of one row (0) / several rows (1).
75. Pappus and involucre of the same length (0) / pappus overtopping the involucre (1).
76. Pappus erect to erect-patent after fruiting (0) / patent after fruiting (1).
77. All the pappi of the capitulum florets of similar length (0) / pappi of marginal florets much longer than those of disc florets (1) / internal pappus bristles of marginal florets as long as pappus of disc florets, and external pappus bristles of marginal florets much longer than pappus of disc florets (2).

While studying African taxa of the Mutisieae (Asteraceae), particularly *Dicoma* Cass. (see Ortiz & Rodríguez-Oubiña, 1994, 1996, 1997; Ortiz et al., 1998; Rodríguez-Oubiña & Ortiz, 1995, 1997), I investigated phylogenetic relationships within *Dicoma*, and between this genus and related taxa.

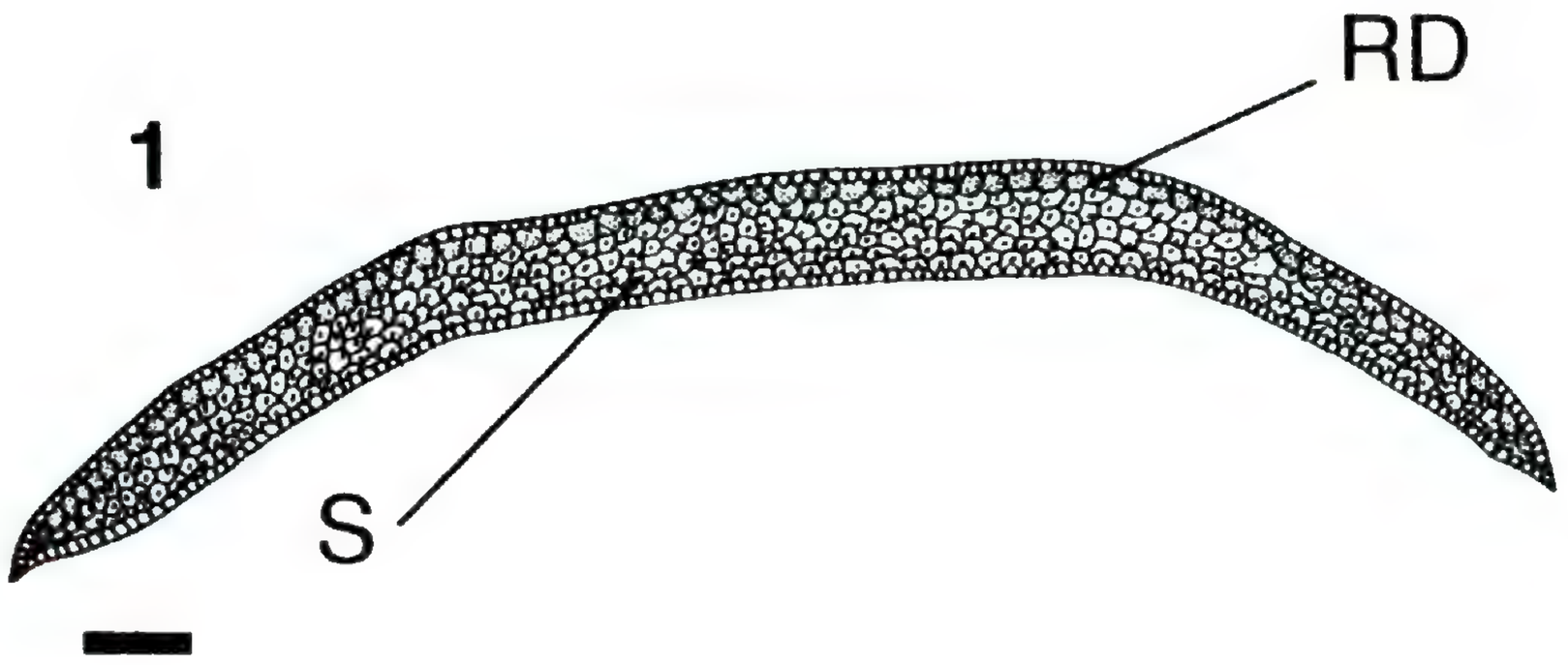
The principal aims of the work reported here were to investigate the relationships between *Dicoma* and other genera and the phylogenetic relationships within *Dicoma*, with the aims of identifying generic and infrageneric subdivisions and of assessing the validity of the subdivisions previously proposed by Lessing (1830, 1832), De Candolle (1838), Harvey (1865), Hoffmann (1893a), Wilson (1923), and Pope (1991) (see Table 1). Another objective was to obtain preliminary information about the geographical origin and evolution of different species groups within *Dicoma*.

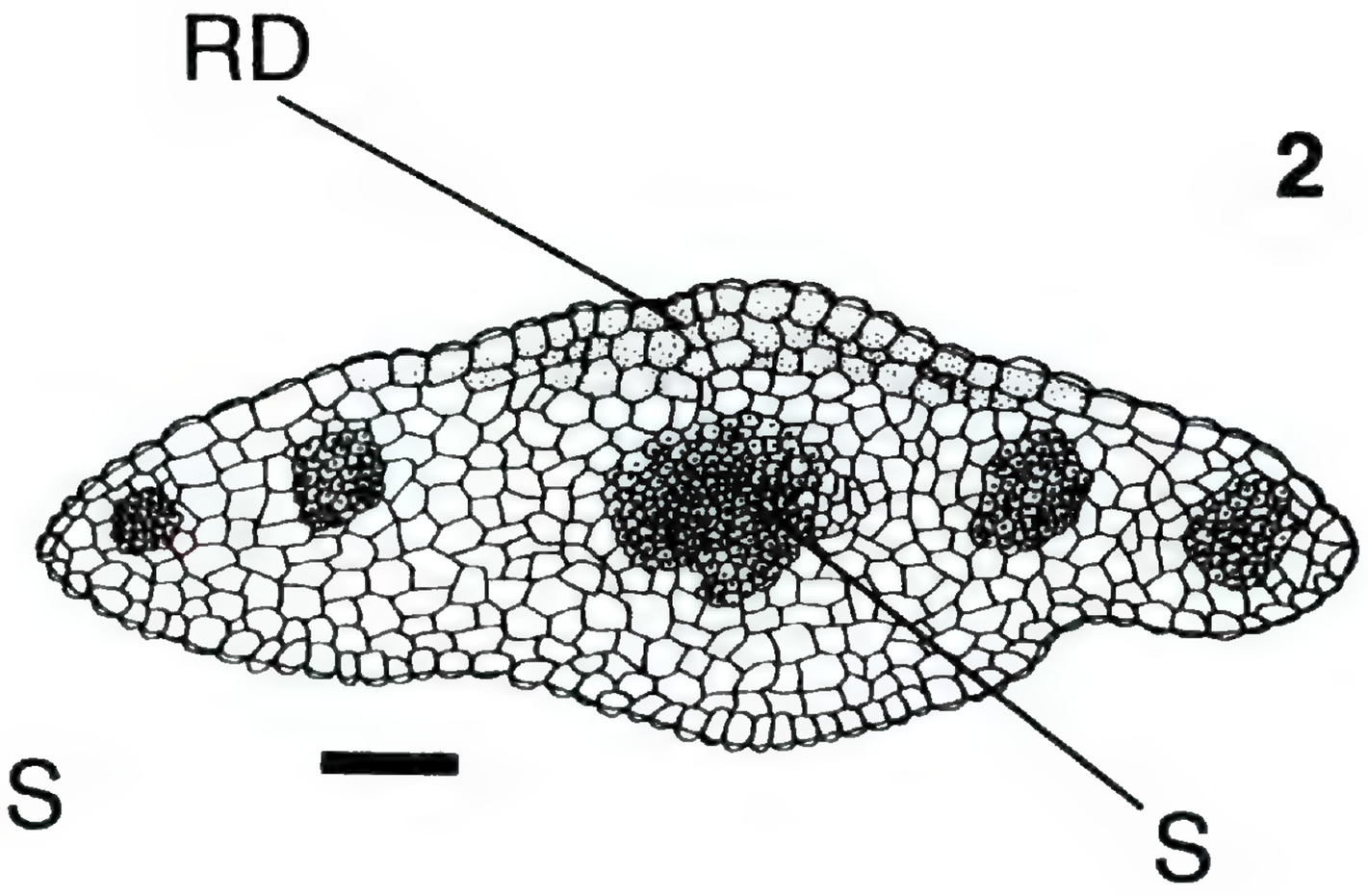
Materials and Methods

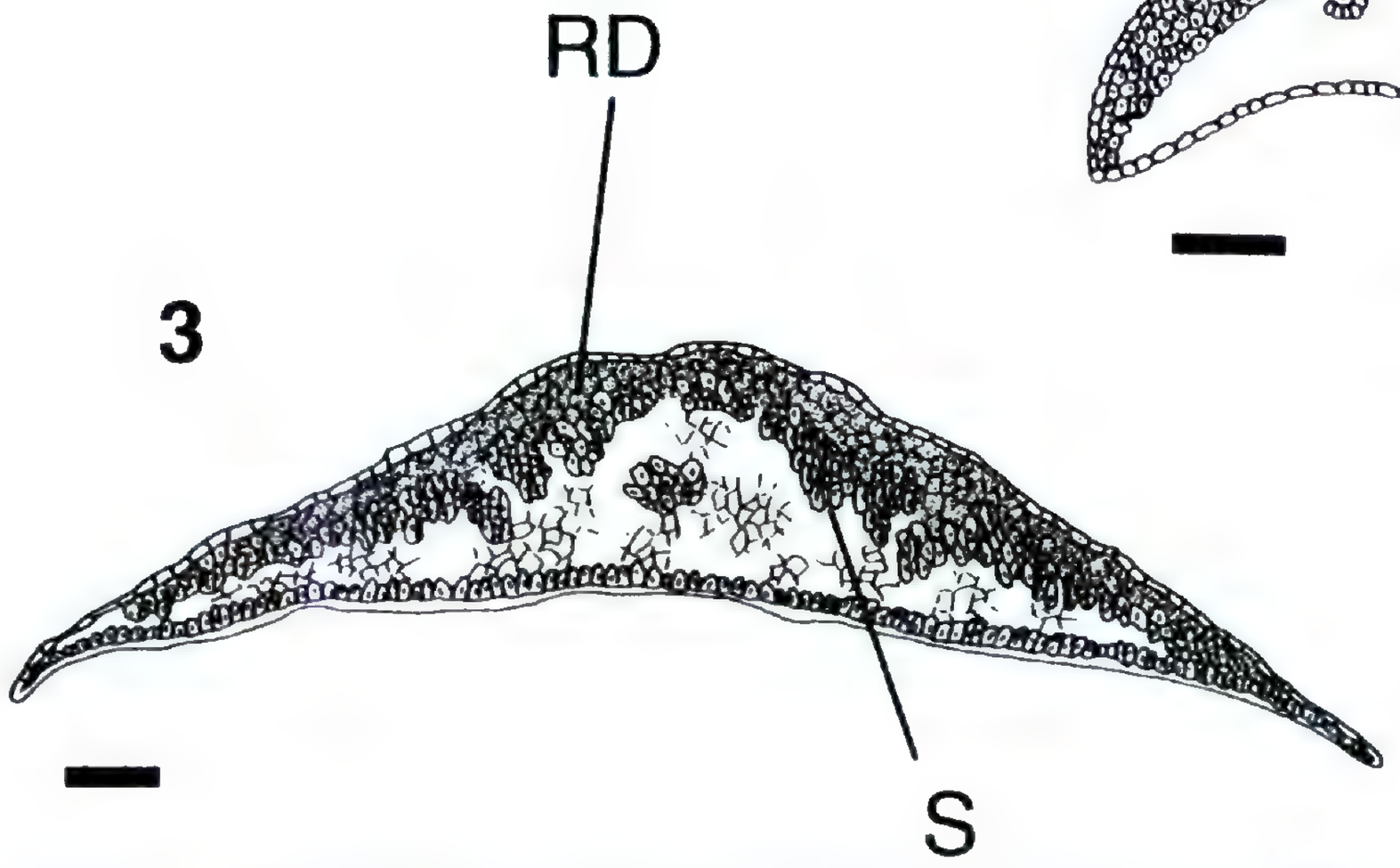
The phylogenetic analysis was based on morphological and anatomical study of specimens from the BM, COI, K, MO, and S herbaria. A number of species of *Dicoma* were selected as representative of the various morphological types present within this heterogeneous genus as follows. First, the seven species of the group I denominate the "*D. sessiflora* group" (see Results) are representative of a morphological type observed in a total of 19 species, including *D. auriculata* Hutch. & B. L. Burtt, *D. elliptica* G. V. Pope, and *D. gossweileri* S. Moore. Second, the species *D. niccolifera* Wild. and *D. capensis* Less. are representative of a morphological type observed in 7 species, including *D. macrocephala* DC., *D. cuneneënsis* Wild., and *D. schinzii* O. Hoffm. Finally, *D. anomala* Sond., *D. aethiopica* S. Ortiz & Rodr. Oubiña, and *D. montana* Schwick. are representative of a morphological type observed in some 10 species not considered in the present analysis, including *D. galpinii* Wilson, *D. popeana* S. Ortiz & Rodr. Oubiña, and *D. somalense* S. Moore. In addition, and with the aim of covering as much morphological variability as possible, we included species from all the known sections of the genus (see Table 1).

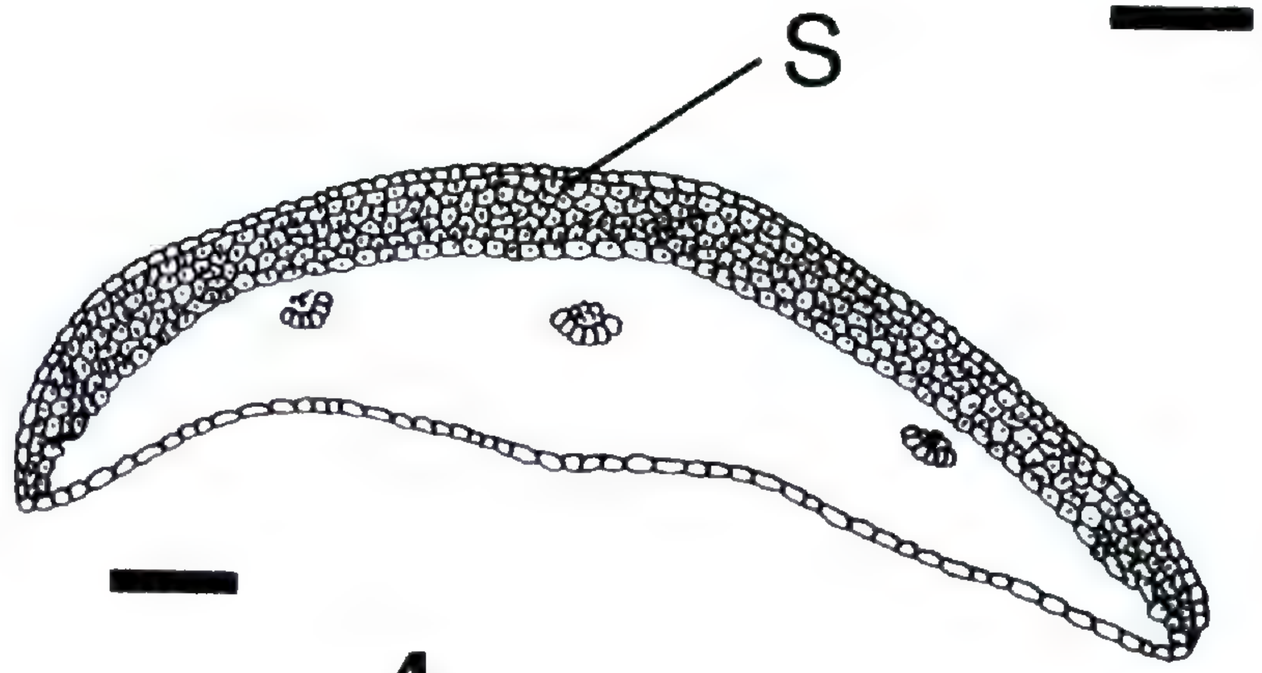
A total of 34 species were included in the study: the single species of *Achyrothalamus*, 24 species of *Dicoma*, 3 species of *Erythrocephalum*, 3 species of *Pasaccardoa*, and 3 species of *Pleiotaxis* (see Table 2). I also studied *Dicoma anomala* Sond., *D. zeyheri* Sond., and *D. capensis* Less. in the field in South Africa. *Gladiopappus vernonioides* Humbert was not included, since I was unable to obtain material of this taxon.

A total of 77 morphological characters were studied (see Table 3). For microscopic examination, floral parts were first boiled in water with a surfactant, then mounted in Hoyer's solution (Anderson, 1954). Phyllary and achene sections were cut by hand with razor blades. Some characters initially considered were excluded from the analysis because they were the same in all species including outgroups, such as endothecial cell wall thickening organization









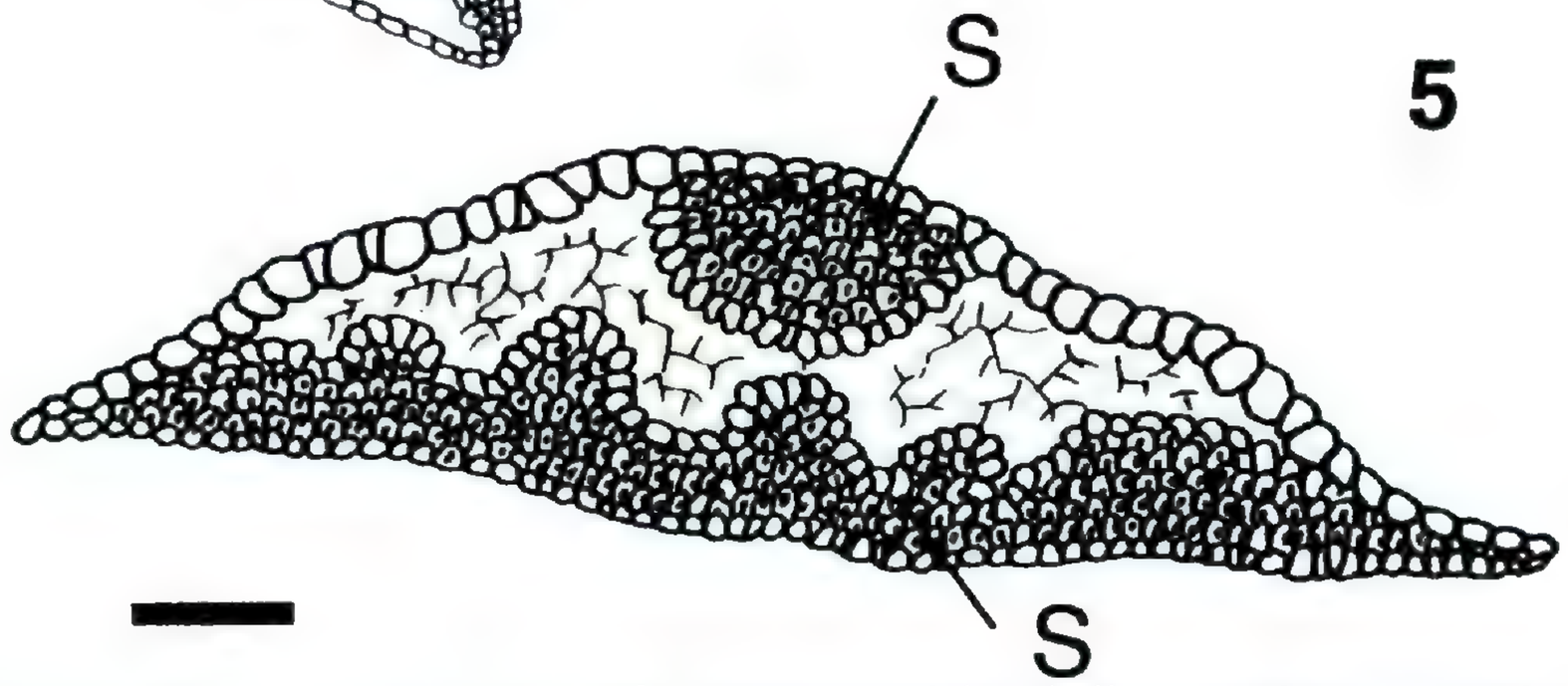


Figure 1. Anatomy of the phyllaries (cross section). —1. Sclerenchymal fibers form a more or less continuous mass occupying the inner part of the phyllary (*Gochnatia picardae* (Urb.) Jimenéz, *Ekman 9280* (S)). —2. The sclerenchymal fibers, though likewise situated in the central part of the phyllary, form clearly distinct groups separated by a very lax parenchyma, which appears to be of aeriferous type (*Oldenburgia grandis* (Thunb.) Baill., *Bayliss 4519* (S)). —3, 4. The sclerenchymal fibers are concentrated toward the abaxial face of the phyllary; in the center, which is basically occupied by parenchyma, there are several vascular bundles reinforced with sclerenchymal fibers (3: *Dicoma carbonaria* (S. Moore) Humbert, *Decary 9263* (MO); 4: *Dicoma saligna* Lawalrée, *Gossweiler 11331* (COI)). —5. The majority of sclerenchymal fibers are either on the adaxial face or on the midrib of the abaxial face, where they protect the vascular bundles (*Dicoma aethiopica* S. Ortiz & Rodr. Oubiña, *Gilbert, Ensermu & Vollesen 7870* (K)). RD: resin ducts. S: sclerenchyma. Scale bar = 0.1 mm.

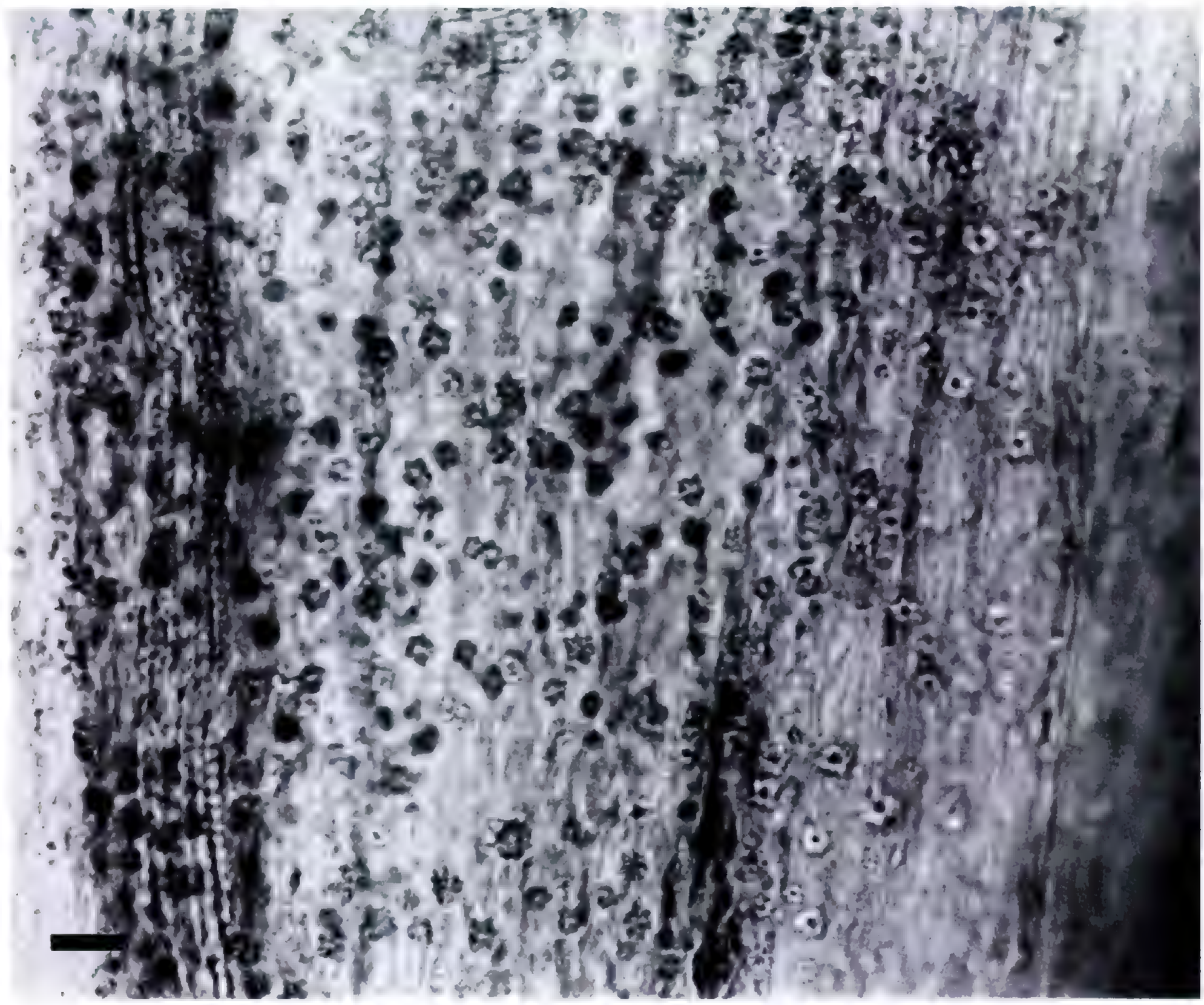


Figure 2. Star-shaped calcium oxalate crystals in the subepidermal cells of the disc corolla of *Dicoma aethiopica* (*Gilbert, Ensermu & Vollesen 7870* (K)). Scale bar = 0.01 mm.

(Dormer, 1962), which was always polarized, and cypsela vein union (Karis et al., 1992), which was always at the base. Some of these characters require explanatory comments, as follows:

Character 8. Many of the species studied commonly show more or less bractiform leaves, denominated "subtending leaves" (Pope, 1991, 1992), at the base of the capitula.

Character 13. Many of the species studied have two dark stripes running along the phyllaries.

Character 14. Examination of cross sections of the phyllaries allows four morphological types to be distinguished, most notably on the basis of the arrangement of sclerenchymal fibers. In the first type, the sclerenchymal fibers form a more or less continuous mass occupying the inner part of the phyllary (Fig. 1-1). In the second type, the sclerenchymal fibers, though likewise situated in the central part of the phyllary, form clearly distinct groups separated by a very lax parenchyma, which appears to be aeriferous (Fig. 1-2). In the third type, the sclerenchymal fibers are concentrated toward the abaxial face of the phyllary; in the center, which is basically occupied by parenchyma, there are several vascular bundles reinforced with sclerenchymal fibers (Fig. 1-3, 1-4). In the fourth type, the majority of sclerenchymal fibers are either on the adaxial face or in the midrib of the abaxial face, where they protect the vascular bundles (Fig. 1-5).

Character 21. *Dicoma carbonaria*, and to lesser extent *D. oleaefolia*, have capitula in which all florets are zygomorphic, with one of the corolla lobes separated from the others by a much deeper incision and without an expanded limb; this morphological type seems transitional between the actinomorphic and the ligulate type and is similar to that seen in certain primitive genera of the subfamily Barnadesioideae, such as *Chuquiraga* Juss., *Dasyphyllum* Kunth, and *Schlechtendahlia* Less. (Hoffmann, 1983a; Cabrera, 1977; Bremer, 1994). "True ray" florets have no adaxial lobes and a 3-lobed lamina.

Character 26. This character was initially studied in ray florets (Baagøe, 1977, 1978), but in my opinion is also informative for species that have disc florets only. Indeed, Hansen (1991) studied epidermal cell cuticle ornamentation in disc florets of species of the various genera considered in the present study (despite the fact that the study in question centers on ray florets). This character has

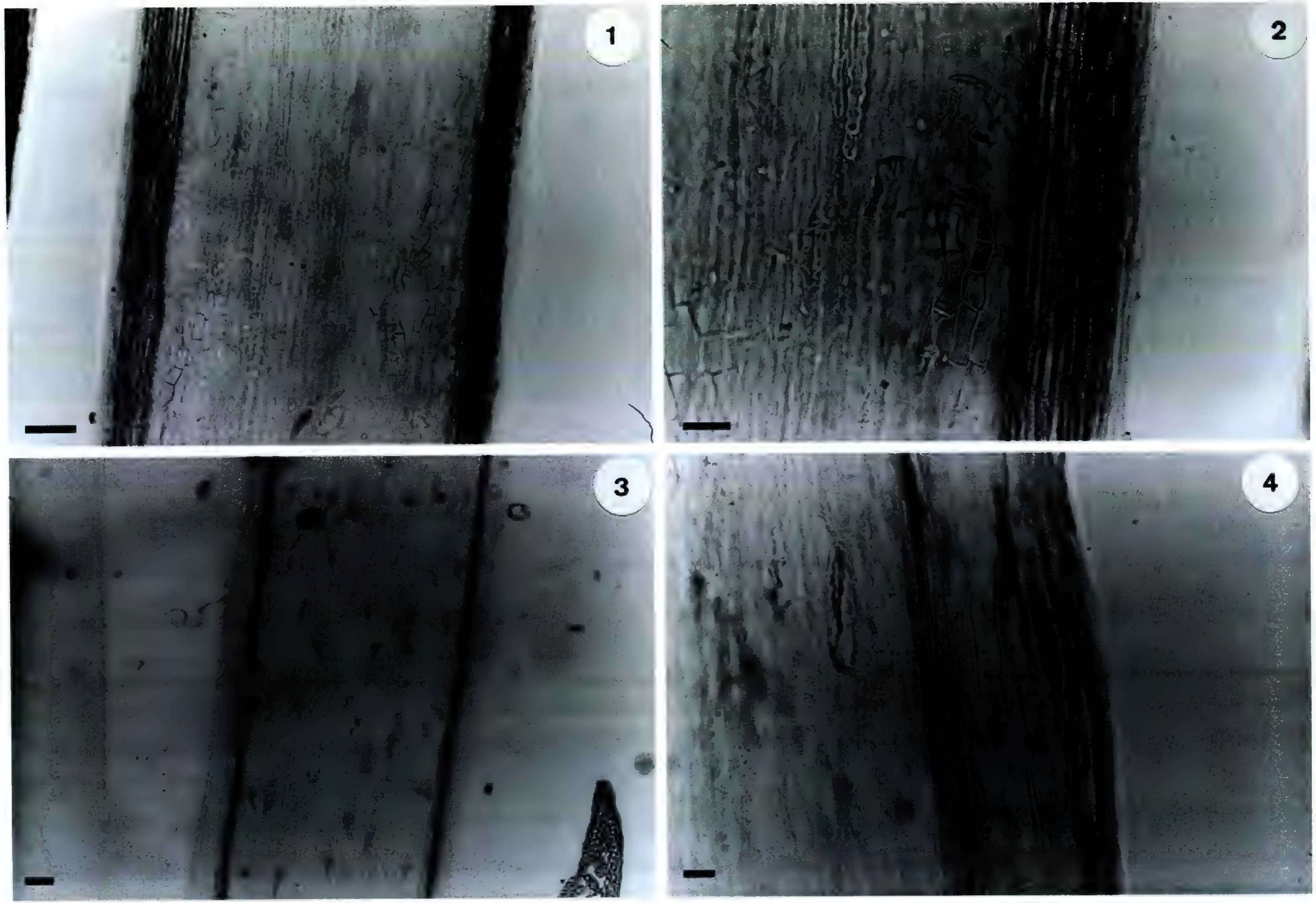


Figure 3. —1, 2. Marginal vascular tissue of the disc corolla lobes of *Dicoma spinosa* (L.) Druce comprising many vessels surrounded by sclerenchymal fibers (*Foucarde 2499* (K)). —3, 4. Submarginal veins of the disc corolla of *Dicoma nachtigalii* O. Hoffm., with fewer vessels and without sclerenchymal fibers (*Pearson 7642* (K)). Scale bar (1 and 3) = 0.05 mm. Scale bar (2 and 4) = 0.01 mm.

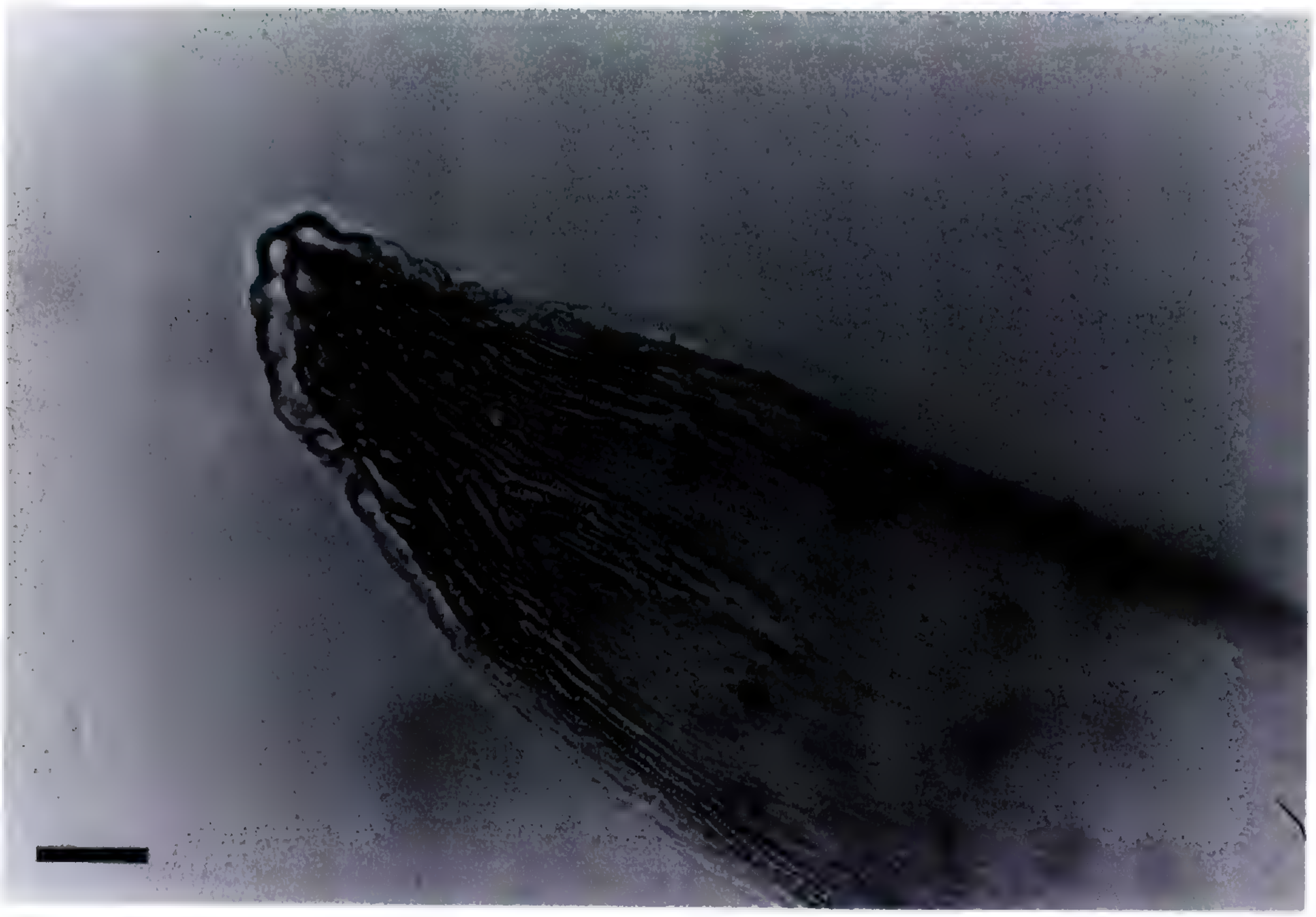


Figure 4. Apex of the disc corolla of *Dicoma aethiopica* (*Gilbert, Ensermu & Vollesen 7870* (K)) with thick-bundled veins. Scale bar = 0.05 mm.

likewise proved useful in the present study, in which most of the species considered lack ray florets. "Intestine-like" ornamentation (Karis et al., 1992) is equivalent to Hansen's "rugose pattern of longitudinal bands" (Hansen, 1991). In the characterization of *Gochnatia* and *Achyrothalamus* I have followed Hansen's (1991) approach, classifying ornamentation in these two genera as "mutisioid" and "senecioid" respectively, though with some reservations, since the distinction between these two typologies was not clearly defined in our material.

Character 28. Some species have long twin glandular hairs with a small apical gland on the corolla (see Karis et al., 1992: 418, fig. 4H). In the rest of the species considered the disc corolla hairs

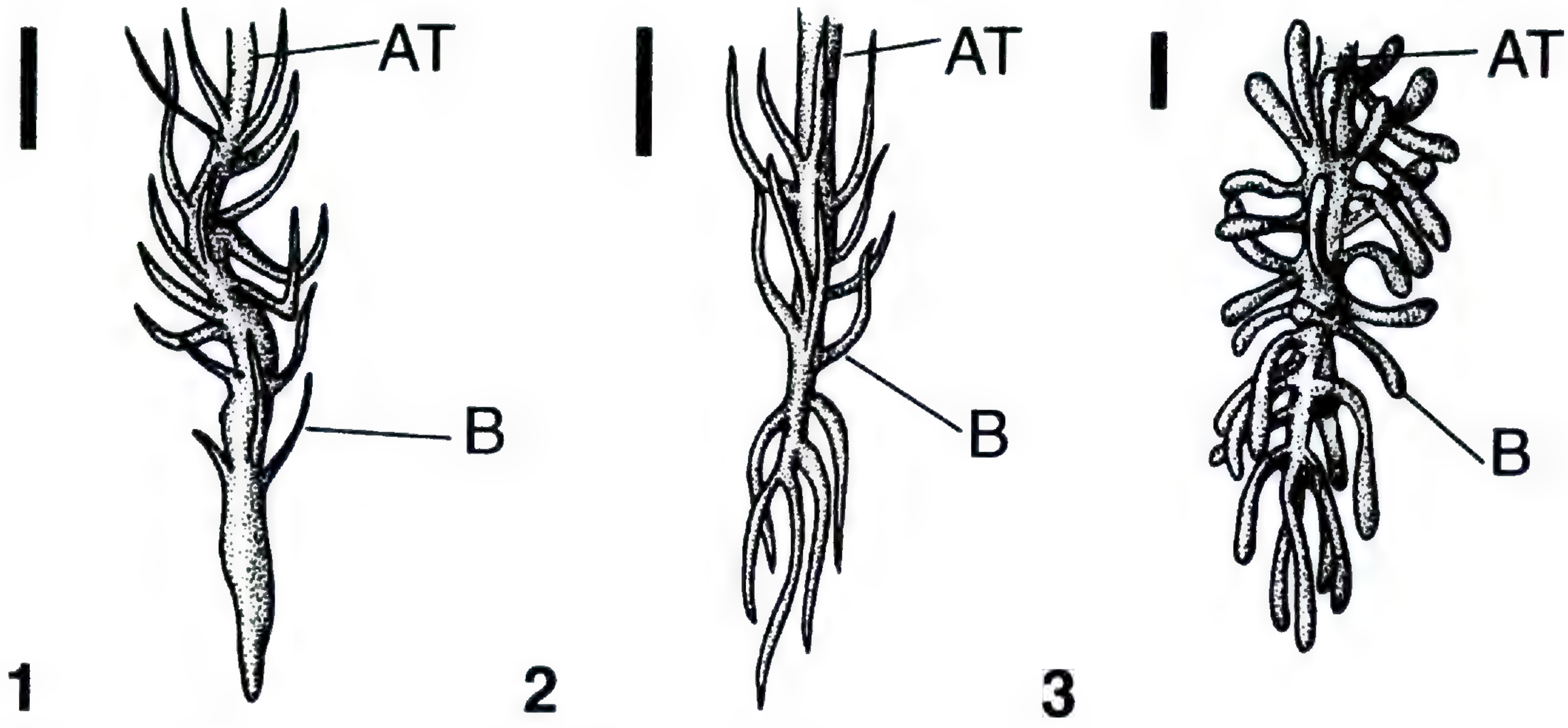


Figure 5. Distal part of the anther tails. —1. Anther apices lack antrorse ramifications and the apex of the anther tail ramifications is acute (*Dicoma saligna, Gossweiler 11331* (COI)). —2. Anther apices have antrorse ramifications and the apex of the anther tail ramifications is acute (*Dicoma tomentosa* Cass., *Nordenstam 2454* (S)). —3. Anther apices have antrorse ramifications and the apex of the anther tail ramifications is obtuse (*Dicoma grandidieri* (Drake) Humbert, *Capuron 18643* (K)). AT: anther tail. B: ramifications. Scale bar = 0.05 mm.

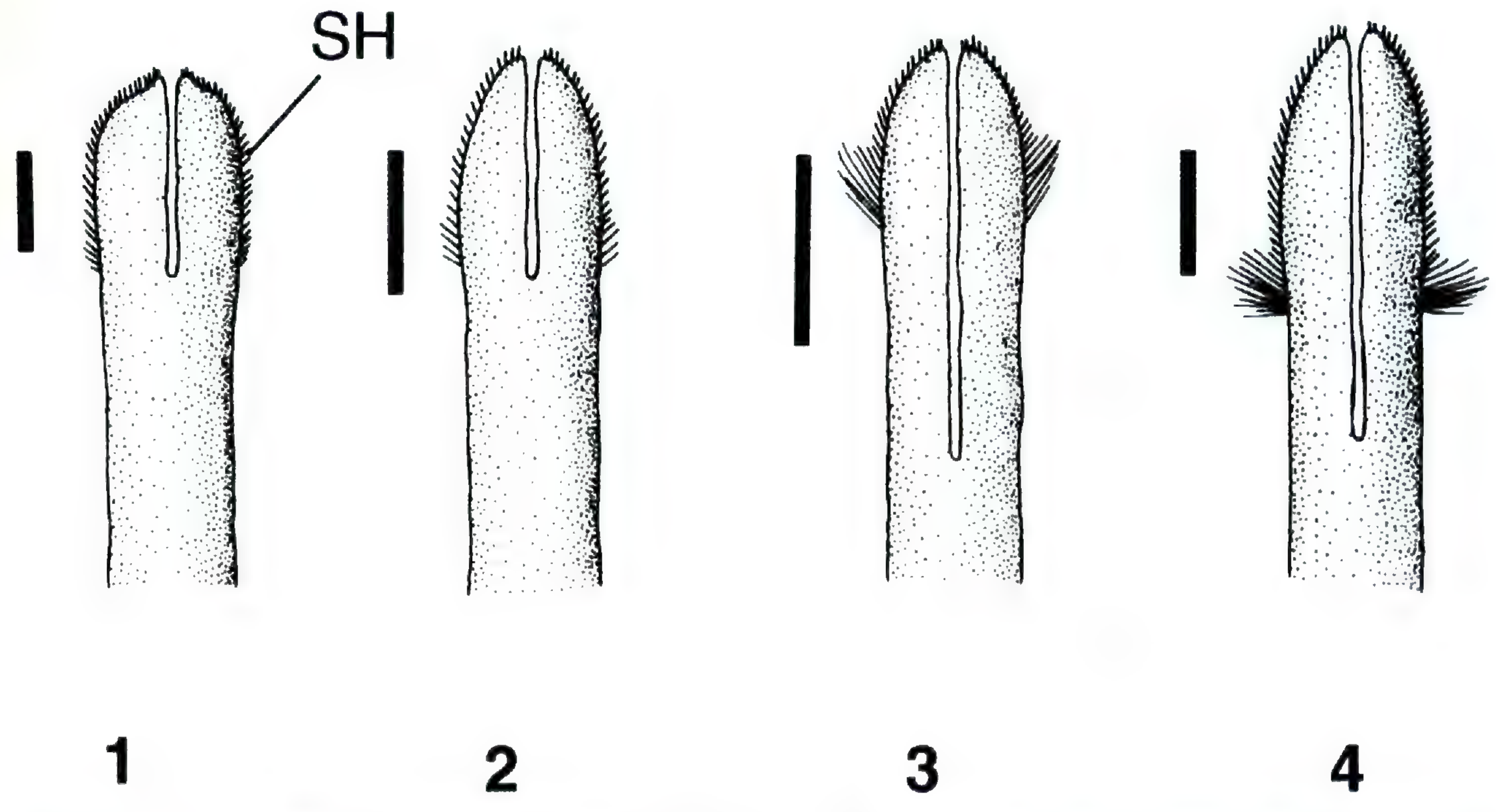


Figure 6. Apical part of the style with sweeping hairs. —1. Short style branches with sweeping hairs of similar length reaching the bifurcation (*Oldenburgia grandis, Bayliss 4519* (S)). —2. Short style branches with a sub-basal group of sweeping hairs slightly longer than the rest, these almost reaching the bifurcation (*Dicoma sessiliflora* Harv. subsp. *sessiliflora, Wingfield 4416* (K)). —3. Long style branches with sweeping hairs in a subapical tuft, with a sub-basal group of sweeping hairs conspicuously longer than the rest (*Dicoma bangueolensis* Buscal. & Muschl., *Beckett 242* (K)). —4. Long style branches with a long group of sweeping hairs not reaching the bifurcation, these with a sub-basal group of sweeping hairs conspicuously longer than the rest (*Dicoma tomentosa, Nordenstam 2454* (S)). SH: sweeping hairs. Scale bar = 0.5 mm.

are "short glandular hairs" with a large apical gland (see Karis et al., 1992: 418, fig. 4I). Rarely (either in the first or second type) "twin hairs" are present that are basically similar to the non-myxogenic short ovoid twin hairs of Karis et al. (1992: 418, fig. 4B), which are very short and which lack a conspicuous apical gland.

Character 29. Disc corolla simple hairs are long simple hairs with one or two basal cells (see Karis et al., 1992: 419, fig. 5E).

Character 32. The marginal vascular tissue of the lobes of the corolla of disc florets is thick in some taxa, made up of many vessels and surrounded by sclerenchymal fibers (Fig. 3-1 and 3-2). In the remaining taxa, the marginal veins are narrow, comprised of fewer vessels and without sclerenchymal fibers (Fig. 3-3 and 3-4).

Character 34. Some species show accessory veins of variable length in the corolla lobes, running more or less parallel to the marginal/submarginal veins.

Character 35. In some species, the veins of the disc corolla lobes form a highly characteristic dense bundle (Fig. 4) (see Karis et al., 1992).

Character 36. In most of the species studied, the stamen filaments are inserted at the tube/throat junction. In *D. carbonaria* and *D. oleaefolia*, by contrast, the stamen filaments are inserted practically at the base of the corolla. The presence of two clearly distinct states is not in agreement with Karis (1993), who reported that this character varies little within each of the tribes of the subfamily Asteroideae (except in the tribe Astereae) and is thus of considerable taxonomic value at the tribal level.

Character 44. *Dicoma carbonaria* and *D. oleaefolia* share an interesting characteristic, the tails of adjacent pairs of anthers being joined, with their ramifications or hairs interwoven. The anthers of the remaining species are clearly separated.

Character 45. In many taxa the apices of the anther tails show characteristic antrorse ramifications, while the ramifications of the rest of the anther tail are always retrorse (Fig. 5-2 and 5-3). In other taxa, however, such apical ramifications are absent (Fig. 5-1).

Character 55. In several taxa the stylar sweeping hairs reach (or almost reach, or extend beyond) the point of bifurcation of the style branches. In most of the other species considered, the stylar sweeping hairs form a short subapical tuft that Hansen (1991) and Bremer (1994) considered characteristic of the species of the "*Dicoma* group." In

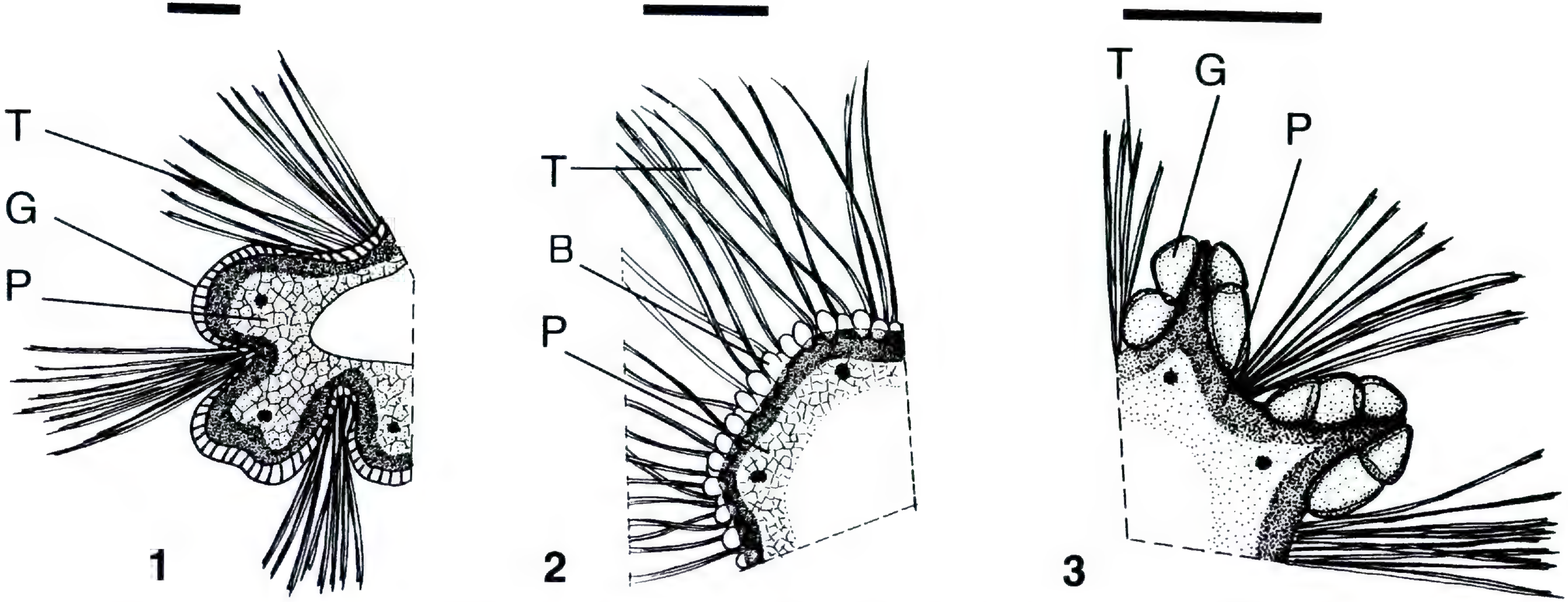


Figure 7. Cross section of the cypsela of *Dicoma* —1. Cypsela ribbed, with superficial glands in a continuous layer, and twin hairs located between the ribs with base not bulbous-glandular (*D. carbonaria, Chauvet 359* (K)). —2. Cypsela not ribbed, without superficial glands and with twin hairs located all around the cypsela and with bulbous-glandular base (*D. saligna, Gossweiler 11331* (COI)). —3. Cypsela ribbed, with superficial glands on the ribs, and twin hairs located between the ribs and with base not bulbous-glandular disposed between the ribs (*D. tomentosa, Nordenstam 2454* (S)). B: base of the twin hairs bulbous-glandular. G: superficial glands. P: pericarp. T: twin hairs (see text). Scale bar = 0.25 mm.

Figure 8. Twin hairs with bulbous-glandular base on the cypsela of *Dicoma saligna* (*Gossweiler 11331* (COI)). Scale bar = 0.1 mm.

Dicoma tomentosa and *D. welwitschii*, a third pattern is observed: the sweeping hairs extend from close to the apex toward the bifurcation, but do not reach it.

Character 59. Cypselas of neuter marginal florets are rudimentary and may or may not have a rostrum.

Character 62. Many of the studied taxa have unicellular cypsela-surface glands containing a resinous material. These may be located between the ribs on the rib walls (Fig. 7-3), between the ribs in the intercostal grooves, or forming a continuous layer around the cypsela (Fig. 7-1), as in the *D. carbonaria* group. *Dicoma fruticosa* shows a morphology intermediate between types 1 and 2, and was coded as inapplicable for this character.

Character 63. Simple twin hairs have the two cells joined, and bifurcate twin hairs have the two cells separated (Karis et al., 1992: 418, fig. 4A).

Character 65. In some species the cypsela twin hairs have a conspicuously bulbous glandular base that contains a resinous material (Fig. 7-2 and 8). In other species the base of the cypsela twin hairs may be somewhat bulbous but is never glandular.

Character 66. In *Pasaccardoa grantii* and *P. jeffreyi*, the twin hairs of the lower part of the cypsela are thicker than the rest; in the remaining species all hairs of the cypsela are similar.

Character 67. Biseriate glands are composed of two rows of cells and a collapsed apical head, and occur in the cypselas of many of the taxa analyzed. In many cases these are not easy to see and often are situated between the superficial glands (see character 62).

Character 68. Subepidermal calcium oxalate crystals are observed in the immature testa of all species. They are subsequently hidden from view by the epidermis of the mature testa. In some species needle-shaped crystals and square to short-rectangular crystals, without uniform orientation, are present in roughly equal proportion (Fig. 9-1). In others, by contrast, almost all the crystals are narrowly rectangular and oriented in the same direction (Fig. 9-2).

Character 69. The first character state corresponds to the testa type defined by Grau (1980) for *Gochnatia* (testa with lateral and basal walls of the epidermal cells strengthened). Similar but not identical morphologies are observed in *Pasaccardoa grantii*, *P. jeffreyi*, and some species of *Dicoma*: in these cases the testa morphology is similar to the *Gochnatia* type, but the cells of the epidermis are of irregular shape, not linear. The second character state is the *Erythrocephalum* type as defined by Grau (1980); in addition to *Erythrocephalum*, the species of *Achyrothalamus* and *Pleiotaxis* fall into this category (basal walls of the epidermal cells of the testa with reinforcements, which give to the cells a lacunose appearance in frontal view). The third character state is the *Dicoma* type as defined

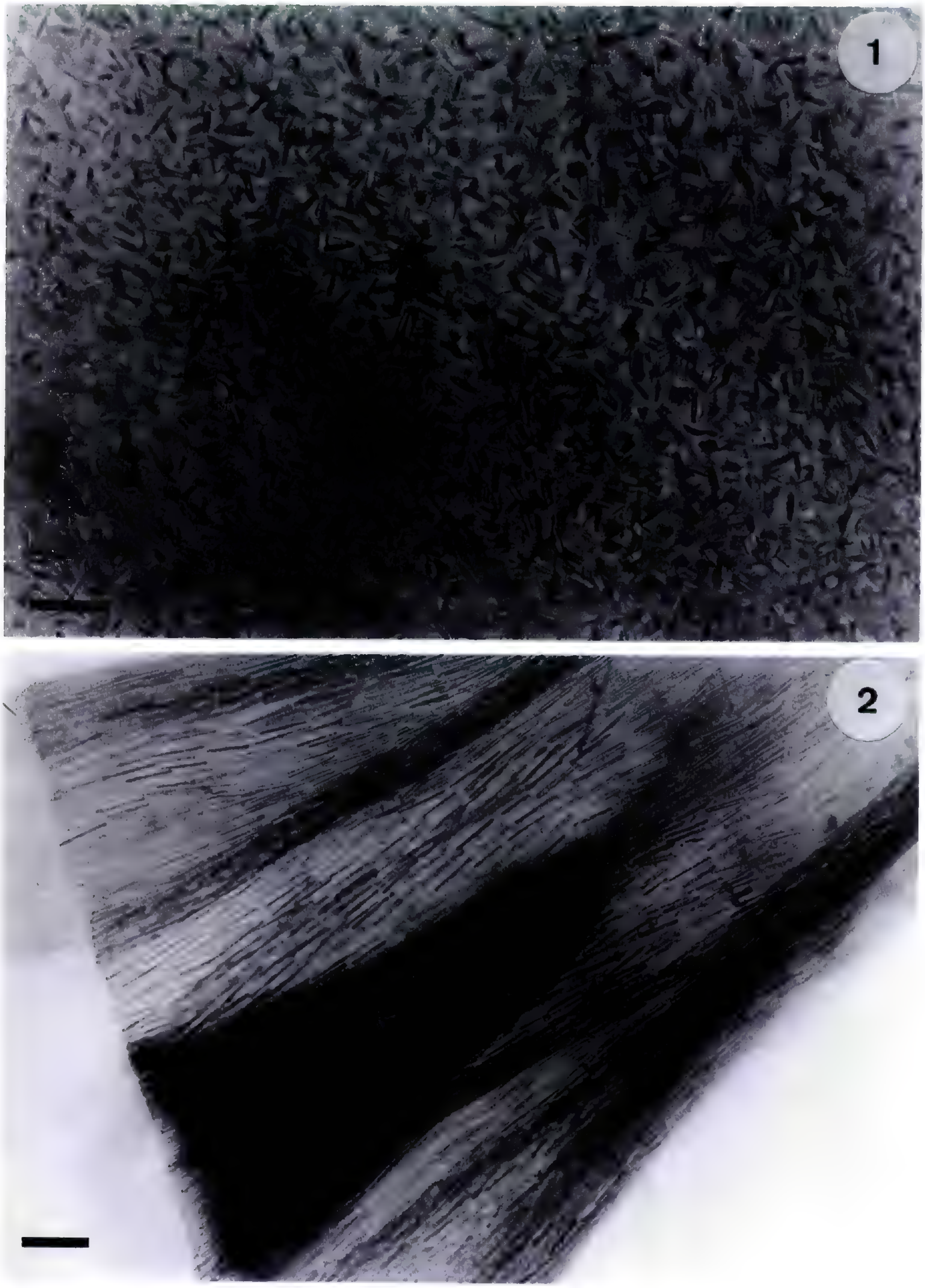


Figure 9. Calcium oxalate crystals in the immature testa (testa subepidermis). —1. Nonoriented needle-shaped crystals and square to short-rectangular crystals in the testa of *Dicoma zeyheri* (*Mogg 16306* (K)). —2. Long-rectangular crystals oriented in the same direction in *Dicoma montana* (*L. E. Codd 8689* (K)) (see text). Scale bar = 0.05 mm.

by the same author (basal walls of the epidermal cells of the testa strengthened with ribs). The fourth character state, observed in *D. welwitschii* and *P. baumii*, is very similar to that observed in the other species of *Pasaccardoa*, except that the epidermal cells of the testa are traversed by thick bands. We have not been able to characterize testa morphology in the *Oldenburgia* material studied; similarly, Karis et al. (1992) were unable to determine whether or not the testa of the species of this genus is "collapsed." The testas of the two species of the *D. carbonaria* group show a morphology similar to Grau's (1980) *Perezia* type (testa with epidermal cells not strengthened), though study of additional material is necessary to confirm this.

Character 70. In some species the lateral and

Table 4. Data matrix. The characters are numbered in accordance with the text and Table 3. Character state unknown is indicated with a question mark, and inapplicable with a dash. Polymorphic taxa are coded with the following symbols: A = (0&1), B = (0&2), C = (1&2), D = (2&3).

	1 11111 11112 22222 22223 33333 33334 44444 44445 55555 55556 66666 66667 77777 77
	12345 67890 12345 67890 12345 67890 12345 67890 12345 67890 12345 67890 12345 67890 12345 67
Gochnatia	00000 000AA 00000 00000 0000A 00000 000A0 0000A 00000 00000 0000- A0000 00000 00000 00000 00
Oldenburgia	00000 00A00 00010 00000 10000 10000 0A000 0000A 00000 00A00 000A0 00000 00B00 010?? 00000 00
Erytrocephalum zambesianum	10100 00100 00000 00001 1001A 20000 01010 00200 10100 11100 11132 00000 00101 01011 1-110 00
Erytrocephalum scabrifolium	10100 001A0 00000 00001 00010 20000 ??010 00100 10100 11100 11132 01000 00101 01011 1-110 00
Erythrocephalum microcephalum	10100 00010 00000 00001 00010 20000 01001 00200 00100 11100 11132 01000 0010? 01011 1-110 00
Achyrothalamus marginatus	10100 000A0 00000 10011 0001A 30000 11010 00200 00100 11100 11132 01000 00101 01011 3---- --
Pleiotaxis pulcherrima	10000 00000 00000 10000 00010 20000 11011 00200 01100 10100 11132 00000 00000 01011 00000 00
Pleiotaxis rugosa	10000 00000 00000 10010 00000 20000 11011 00100 01100 11100 1A132 00000 002-- 01011 00000 00
Pleiotaxis subpaniculata	10000 000A0 00000 10000 000?0 20000 11010 00100 01?00 10100 10030 00000 00000 01011 00000 00
Pasaccardoa jeffreyi	10100 00100 01021 00000 31011 21001 01000 01100 10001 10001 00132 11011 13010 10100 1-000 00
Pasaccardoa grantii	10000 00110 01021 00000 3101A 21001 01000 01100 10001 10001 00132 11011 13010 10100 1-000 00
Pasaccardoa baumii	10000 00000 01021 00000 310?1 21000 ?10A0 01100 10001 10001 00132 11111 12010 01130 1-000 00
Dicoma dinteri	00000 00111 01131 00000 00001 20001 01000 01100 10000 10001 00132 11101 12010 00121 00000 00
Dicoma bangueolensis	00000 00011 01131 00000 00001 20001 01000 01100 00000 10001 00132 11101 12010 00121 20000 00
Dicoma schimperi	10000 00110 11131 00000 00001 20001 01000 01100 10000 10001 00131 11101 12010 00121 00010 00
Dicoma elegans	1A0A0 101A0 011D1 00000 01001 20001 01000 01100 10000 10001 00132 11101 12010 01121 00000 02
Dicoma nachtigalii	00010 0A110 01131 00000 0000A 20001 01001 01100 10000 10001 00132 11101 12010 00121 00000 00
Dicoma fruticosa	00000 00110 01131 00010 31001 20001 ?1001 01C00 10000 10001 0013? 11101 1?010 00121 00000 00
Dicoma montana	00000 00110 01131 00000 00001 20001 01000 01100 10000 10001 00132 11101 12010 00121 00000 00
Dicoma aethiopica	10000 00110 01131 00000 00001 20001 01000 01100 ?0000 10001 00132 11101 12010 00121 00000 00
Dicoma niccolifera	11000 001A0 01131 00000 0100A 20001 11001 00C00 00000 10001 00132 11101 12010 00121 00000 01
Dicoma capensis	11000 00110 01131 000A0 01001 20001 01001 00100 00000 10001 00132 11101 12010 00121 00000 01
Dicoma gillettii	00000 001A0 01A30 00000 00000 20001 01001 01100 10000 10101 00131 11101 12010 00121 00?00 00
Dicoma welwitschii	10000 00100 01021 00A00 000?1 21001 01001 01100 10001 10001 00122 11101 12010 01130 1-000 00
Dicoma tomentosa	10000 01110 11131 00A00 0000A 20001 01000 01100 10000 10001 00122 11101 12010 00121 20000 00
Dicoma anomala	10000 00110 01131 00000 00001 20001 0100? 01100 ?0000 10?01 00112 11101 12010 00121 00000 00
Dicoma picta	00000 00010 01131 00010 31001 20001 0100? 01100 10000 10001 00112 11101 12010 00121 00000 00
Dicoma cana	000A0 10010 10021 01010 0000A 10101 00100 01110 10000 10?0A 00011 11100 00001 00000 00000 00
Dicoma spinosa	00001 00A10 10021 0AA10 0000A ?0111 001A0 01110 10001 10001 00011 11100 1000A 01000 1-000 00
Dicoma grandidieri	00001 001A1 00021 01110 00000 10101 00100 01100 10000 00001 00012 11100 10001 00000 00000 00
Dicoma sessiliflora	100A0 00A00 10021 01110 00000 10100 00100 01100 10001 10001 00012 11100 00001 01000 01000 00
Dicoma zeyheri	100A0 00000 10021 01110 00000 1010A 00100 01100 ?0001 10000 00011 11100 00001 01000 01000 00
Dicoma saligna	10000 001A0 00021 01110 00000 10101 00100 01100 10001 10?00 00011 11100 00001 01000 01000 00
Dicoma relhanioides	00001 00110 00021 0AA10 0000A 10101 00110 01100 10001 10001 00012 11100 10001 01000 1-000 00
Dicoma carbonaria	00010 00111 00020 00000 20100 20010 21000 1A101 00010 10110 00011 01101 11010 01??1 00001 10
Dicoma oleaefolia	00010 00111 00020 00000 20100 20010 21000 10101 00010 10111 00011 01101 110A0 010?1 00001 10

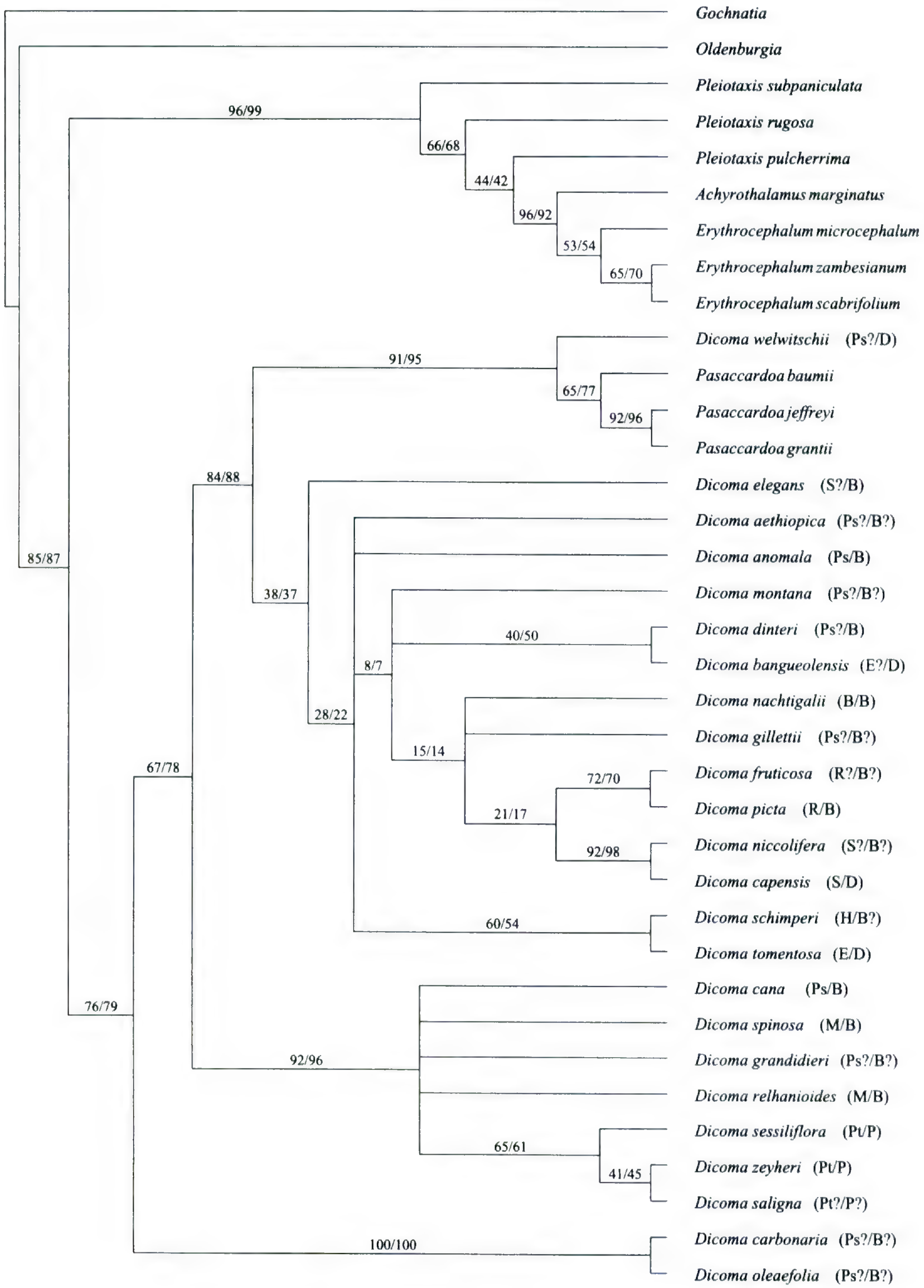


Figure 10. Strict consensus tree of *Dicoma* and related genera, based on the six equally most parsimonious cladograms obtained from analysis of the data in Table 4. The numbers at each node are % jackknife support (before the slash) and % bootstrap support (after the slash). Letters in parentheses after *Dicoma* species indicate the section to which each species belongs. Sections proposed by Hoffman (1893a) (before the slash): S, *Steirocoma*; R, *Rhigiothamnus*; E, *Eudicoma*; M, *Macledium*; H, *Hochstetteria*; Ps, *Psilocoma*; B, *Brachyachaenium*; Pt, *Pterocoma*. Sections proposed

basal walls of the epidermal cells of the testa are strengthened with a highly characteristic morphology, U-shaped in cross section and similar to that of Grau's (1980) *Gochnatia* subgroup. In the remaining species only the basal walls are thickened with ribs.

Character 71. Pappus is only absent in *Achyrothalamus*. In the remaining species it is formed by rather fine bristles, as in *Pleiotaxis* and in many species of *Dicoma*, by bristles and scales as in *D. bangueolensis* and *D. tomentosa*, or by scales only as in *Erythrocephalum*, *Pasaccardoa*, *Dicoma welwitschii*, *D. spinosa*, and *D. relhanioides*.

Character 73. *Erythrocephalum* has a pappus of narrow caducous scales, while in all other species considered the pappus is persistent.

Character 77. In most of the species analyzed the pappi of the different flowers that make up the capitulum are all of similar length. In *Dicoma niccolifera* and *D. capensis*, the pappi of the marginal florets are much longer than those of the disc florets. In *D. elegans*, the internal pappus bristles of the marginal florets are as long as the pappus of the disc florets, while the external pappus bristles of the marginal florets are much longer than the pappus of the disc florets.

Polarization of characters was determined by the outgroup comparison method (Stevens, 1980; Watrous & Wheeler, 1981; Maddison et al., 1984), using *Gochnatia* Kunth and *Oldenburgia* Less. as outgroups. Both genera are of the tribe Mutisieae, and in Karis et al.'s (1992) analysis they are basal to the taxa included in the ingroup. *Gochnatia* is a heterogeneous genus with nearly 68 species (Bremer, 1994), mostly from the Americas, though also Asia. For outgroup comparison, I selected 5 species of *Gochnatia*, representatives of three of the five sections of the genus (*G. amplexifolia* (Gardner) Cabrera, *G. attenuata* (Britton) Jervis & Alain, *G. cordata* Less., *G. microcephala* (Griseb.) Jervis & Alain, and *G. picardae* (Urb.) Jiménez) with similar habit to those of the *Dicoma* group. *Oldenburgia* is a genus of four species endemic to the Cape area in South Africa, from which three species (*O. grandis* (Thunb.) Baill., *O. papionum* DC., and *O. paradoxa* Less.) were selected.

Characters with only two states of which one was autapomorphic were not included in the analysis.

Wagner parsimony analysis of the data matrix (Table 4) was performed on a PC with the aid of the program PAUP* 4.0 (Swofford, 1998). Cladograms were generated using a heuristic search with the TBR (tree bisection-reconnection) branch-swapping algorithm with random additions (100 replicates). Support values for each clade were obtained by jackknife analysis (100 replicates) (Farris et al., 1996) and bootstrap analysis (Felsenstein, 1985). Successive weighting (Farris, 1969) was performed for generating cladograms where the relative weight of homoplasious characters was reduced. All multistate characters were treated as nonadditive. Some characters were coded as polymorphic for some terminals. In the data matrix, unknown character states were indicated with "?", and inapplicable character states were indicated with a dash.

Results

The cladistic analysis yielded six equally most parsimonious cladograms, each 186 steps long, with a consistency index (CI) of 0.513, and a retention index (RI) of 0.82, including in both cases the only uninformative character of the matrix. The six cladograms showed only minor differences. One of the six cladograms is shown in Figure 11.

Analysis with successive weighting gave two cladograms (Fig. 12), with identical major-clade topology to the six equally most parsimonious cladograms (and the corresponding strict consensus tree) obtained without successive weighting. However, the two successive-weighting cladograms differed from the no-weighting strict consensus tree in the internal topology of the major clades.

In the strict consensus tree (Fig. 10), the first division split (a) the first clade, comprising the genera *Pleiotaxis*, *Achyrothalamus*, and *Erythrocephalum*, from (b) the genera *Dicoma* and *Pasaccardoa*. The second division split (a) the Madagascan endemics *D. carbonaria* and *D. oleaefolia* (hereinafter referred to as the *D. carbonaria* group) from (b) the remaining species of *Dicoma* and *Pasaccardoa*. The third division split (a) the seven species of the *Dicoma* sections *Pterocoma* DC., *Macledium* Less., and *Psilocoma* Harvey, hereinafter referred to as the *D. sessiflora* group, from (b) the remaining species of *Dicoma* plus the three species of *Pasaccardoa*. The fourth division gave the last two major clades, one comprising *Dicoma welwitschii* and the *Pasaccardoa*

←

by Wilson (1923) (after the slash): D, *Dimorphae*; B, *Barbellatae*; P, *Plumosae*. Question marks indicate that the species in question has not been assigned to any section before this paper (see Table 1).

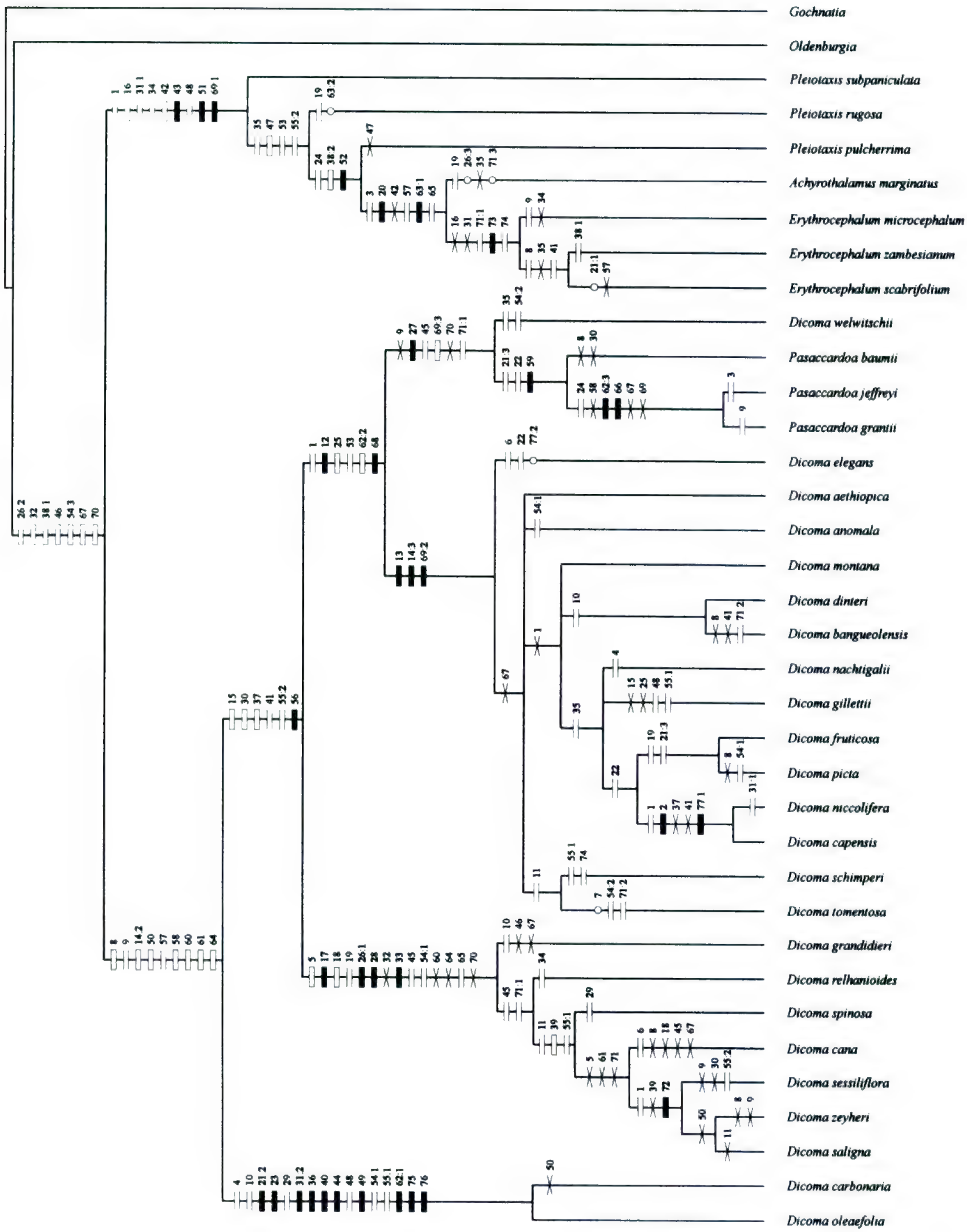


Figure 11. One of the six equally most parsimonious cladograms of *Dicoma* and related genera illustrating the character distribution. Character numbering follows that shown in Tables 3 and 4. ■ synapomorphy (c = 1). □ synapomorphy (c < 1). ○ autapomorphy. = parallelism. X reversal.

species, the other comprising the species I refer to as the *D. tomentosa* group.

In general, the five major clades revealed by the analysis are adequately supported by jackknife and bootstrap values (see Fig. 10), and the results of the analysis can therefore be considered sufficiently reliable to provide a basis for elucidation of phylogenetic relationships among the genera and spe-

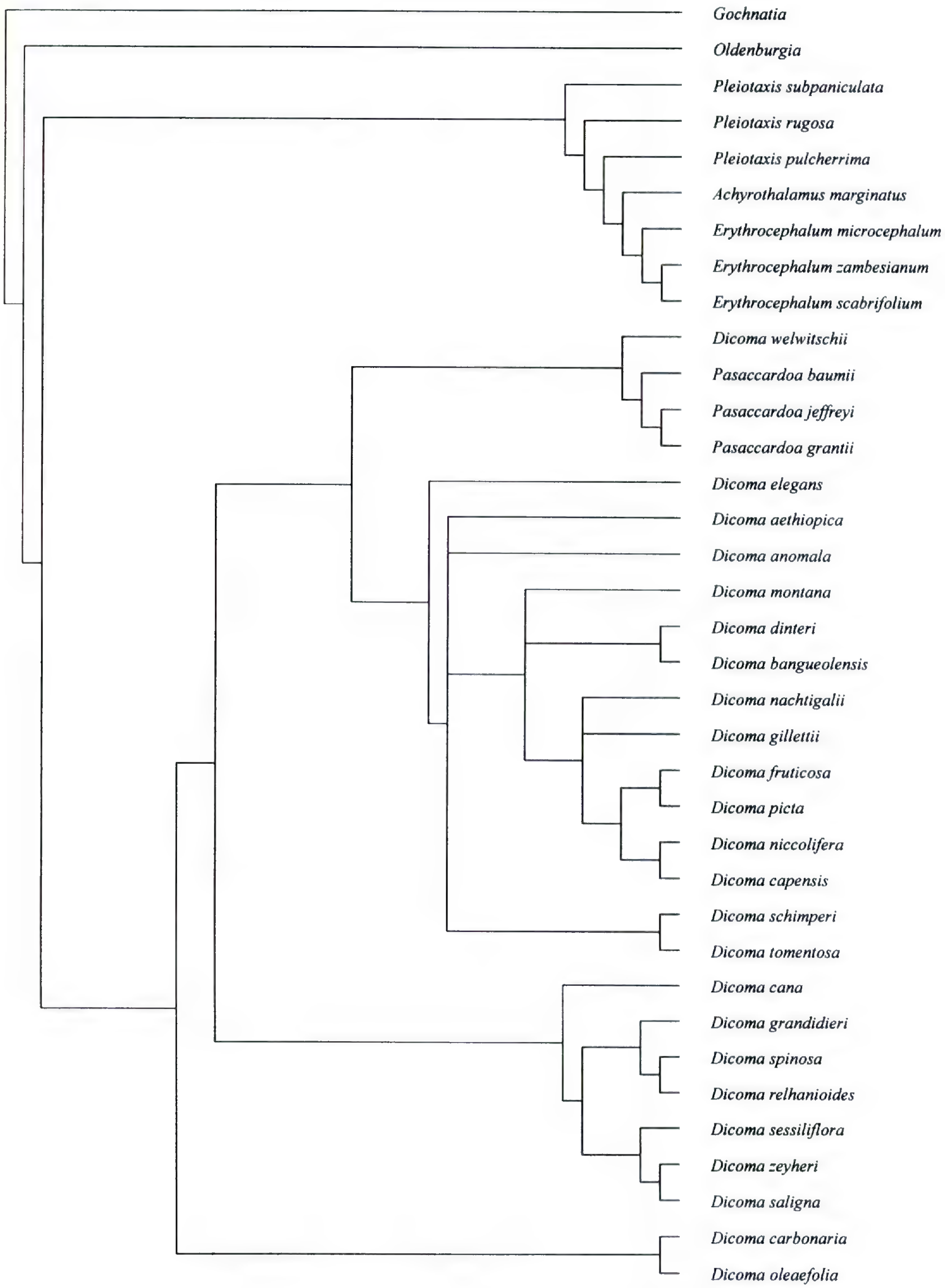


Figure 12. One of the two equally most parsimonious cladograms of *Dicoma* and related genera obtained in the successive weighting analysis.

cies considered. Only the clade corresponding to the *D. tomentosa* group had low jackknife and bootstrap support.

Discussion

The results of the analysis reported here contradict those of Karis et al. (1992), in that *Erythrocephalum* and *Pleiotaxis* appear to be more phylogenetically advanced than *Dicoma*.

The analysis indicates that the genera *Pleiotaxis*, *Achyrothalamus*, and *Erythrocephalum* constitute a monophyletic group (see Fig. 10), the members of which share a number of apomorphic characters, some of which are exclusive (such as anther tails with subrounded to subacute apex, or style branches separated). Within this group, *Erythrocephalum* and *Pleiotaxis* appear to be paraphyletic. Nevertheless, confirmation of this would require a cladistic analysis of these three genera alone, with consideration of all or most of the species included within them; the present study focused on the species of *Dicoma* and on the characters relevant to the taxonomy of this genus. Such an analysis should try to identify other characters that although of little diagnostic value in the present study might be of value in an analysis of these three genera alone. Despite these reservations, it is of interest that *Achyrothalamus* and *Erythrocephalum* showed only minor differences (characters 16, 19, 26, 31, and 71); of these characters, the absence of a pappus (character 71) is the most widely used to distinguish between *Achyrothalamus* and *Erythrocephalum* (which has a caducous pappus). In this connection, our observations show that bifid twin hairs are present on the cypsela of *Achyrothalamus*, and that these hairs are apparently identical to those previously considered to be diagnostic of *Erythrocephalum* and *Hyaloseris* (Karis et al., 1992). The presence of these hairs in *Achyrothalamus* does not seem to have been taken into account when deciding to consider this taxon as a separate genus, presumably because they have not previously been detected: there is no mention of them in the original description of the genus (Hoffmann, 1893b), and Bremer (1994) described the cypselas of *Achyrothalamus* as glabrous.

The clade comprising *Dicoma carbonaria* and *D. oleaefolia* (the "*D. carbonaria group*") (see Fig. 11) is well defined and clearly separated from the remaining species of *Dicoma*. Important apomorphic character states shared by these species include (a) capitulum of all florets zygomorphic [since the subfamily Barnadesioideae is one of the most primitive groups within the Asteraceae (Bremer, 1994), the character state considered apomorphic, as observed in *D. carbonaria* and *D. oleaefolia*, should in a wider context probably be viewed as a reversion to a more primitive type; like Cabrera (1959), Bremer (1987), and Karis et al. (1992), I consider the most primitive state to be that in which all florets are actinomorphic] (character state 21.2), (b) disc corollas much longer than involucre (23.1), (c) disc corolla veins separated as far as the corolla base (31.2), (d) stamens inserted close to the base of the corolla [the marginal nerves of the corolla remain separated to the base of corolla, as in certain species of the genus *Stenopadus*, of the Guayana Highlands (Carlquist, 1957; Bremer, 1994), one of the most primitive genera or possibly the most primitive genus (Hansen, 1991; Karis et al., 1992; Bremer, 1993a, b, 1994) of the Mutisieae] (36.1), (e) anther projected beyond the corolla, (f) anther tails of contiguous anthers joined (44.1), (g) style with four veins (49.1), (h) superficial cypsela glands disposed in a continuous layer all around the cypsela (62.1), (i) pappus much longer than involucre (75.1), and (j) pappus bristles patent after fruiting (76.1). All of these synapomorphic characters, some relating to relevant aspects of floral morphology, together with a series of plesiomorphic characters not observed in the remaining species of *Dicoma* (such as the presence of resin ducts, the absence of star-shaped calcium oxalate crystals in subepidermal cells of the corolla, and the presence of narrow style branch nerves), support the consideration of the *D. carbonaria* group as a clearly distinct clade, and raise the possibility that these two arborescent Madagascan endemics should be considered as a separate genus. This would entail resurrecting the genus *Cloiselia* S. Moore, described by this author (Moore, 1906) for *C. carbonaria* (*D. carbonaria*). I am unaware of other species of *Dicoma* with floral characteristics like those observed in the *D. carbonaria* group; certain of these characters, as mentioned above, are clearly primitive (corolla ligulate but without expanded limb, stamens inserted close to the base of the corolla, abundant long simple hairs on the corolla surface). These characters suggest a relationship with the genera of the Barnadesioideae (notably *Dasyphyllum*, *Chuquiraga*, and *Schlechtendahlia*) and the likewise primitive South American Mutisieae genus *Stenopadus* (see Cabrera, 1977; Bremer, 1994). This raises the possibility that the relationship between the African Mutisieae and the more primitive South American representatives of this tribe is perhaps closer than is currently admitted. This would be consistent with the hypothesis of Bremer (1993a, b, 1994), whereby the Asian species of Mutisieae were derived from

the South American species as a result of westward spread across the Pacific. It is also possible that the primitive characteristics of the *D. carbonaria* group reflect the isolation and protection from competition that has affected the evolution of many other plant and animal lineages on Madagascar. Of the *Dicoma* species not considered in the present study, the only taxon showing some degree of external morphological similarity to the species of the *D. carbonaria* group is *D. incana* (Bak.) O. Hoffm., likewise arborescent and likewise endemic to Madagascar; note, however, that floral morphology characters in this species follow the normal pattern for the genus.

The next clade (see Fig. 11) comprises two clades, one of them including the seven species that I refer to as the *D. sessiliflora* group. That this clade is genuinely representative of a phylogenetic entity is supported by a series of relevant non-homoplastic (CI = 1) synapomorphic characters, including innermost phyllaries more or less entirely scarious (17.1), and corolla with long twin glandular hairs (28.1). Other synapomorphic characters are homoplastic (CI < 1), due either to parallel evolution (e.g., phyllary anatomy, marginal veins of the disc corolla lobes in marginal or almost marginal position, base of the twin hairs of the cypsela very conspicuously bulbous-glandular) or to reversions to the plesiomorphic state (e.g., innermost phyllaries shorter than the contiguous outer series, no antrorse branches at the apex of the anther tails). Plesiomorphic characters shared by the members of this group include marginal veins of the disc corolla lobes broad (32.0), cypsela ribs absent or very slender (60.0), and twin hairs all around the cypsela (64.0). Confirmation that the *D. sessiliflora* group constitutes a distinct entity within *Dicoma*, or possibly a separate genus, will require a more detailed analysis considering all the species in the group. It is worth noting that some of the species of this group are more adapted to woodland conditions than those of the *D. tomentosa* group (which, together with *D. welwitschii* and *Pasaccardoa*, constitutes its sister group); the species of the *D. tomentosa* group, though sometimes occurring in woodland habitats, are generally better adapted to dry, open sites, and even sub-desert or desert habitats (see Lisowski, 1991; Pope, 1992).

The members of this latter clade (see Fig. 11) share a number of apomorphic characters including (a) a conspicuous phyllary midrib (12.1), (b) disc corolla lobes recurved at apex [the corolla lobes have a recurved apex, with the apices of the anthers and the pistil exserted; this may reflect the adaptation of most species of this group to desert or sub-desert environments in which pollinators are very scarce, so that there is selection in favor of at least partial anemophily (see Whitehead, 1969; Lane, 1996)] (25.1), (c) cypsela ribs conspicuously strong (60.1), (d) cypsela with twin hairs between the ribs (as also observed in the *D. carbonaria* group, and presumably reflecting parallel evolution) (64.1), and (e) immature testa (subepidermal layer of the testa) with long-rectangular crystals oriented in the same direction (68.1). This group splits into two clades: one in which *D. welwitschii* appears as a sister group of the three species of *Pasaccardoa*, and another comprising the *D. tomentosa* group. The latter might be referred to as *Dicoma* s. str., since it contains *D. tomentosa*, the type species of the genus.

In view of the topology of the cladogram (Figs. 10–12), one possible approach would be to transfer the species of the genus *Pasaccardoa* to *Dicoma*. Alternatively, *Pasaccardoa* could be maintained, in view of its various synapomorphic characters, one non-homoplastic (cypsela of the marginal florets with rostrum) and others shared with other species but not with the members of the *D. tomentosa* group (disc corolla tube abruptly dilating into limb, margin of the disc corolla lobes conspicuously sclerified, no antrorse branches at the apex of the anther tails, pappus of scales). *Pasaccardoa* also has a number of relevant plesiomorphies including presence of a cylindrical disc floret cypsela, and *Gochnatia*-type testa. This view is supported by the fact that *D. welwitschii*, which the analysis indicated to be a sister group to *Pasaccardoa*, shows a number of character states different from those of the *D. tomentosa* group, some apomorphic (phyllary sclerenchymal fibers concentrated on the abaxial face, margin of the lobes of the disc corollas conspicuously sclerified, absence of antrorse branches at the apex of the anther tails, testa of *D. welwitschii* type, pappus of scales) and some plesiomorphic (capitulum wider than 20 mm, absence of dark stripes along the phyllaries, and lateral and basal walls of testal epidermal cells strengthened). Some of these characters are shared with the species of *Pasaccardoa*. However, the inclusion of *D. welwitschii* within *Pasaccardoa* does not seem to be justifiable, in view of the marked differences with respect to the species of this genus, notably *P. grantii* and *P. jeffreyi*. These two species, in addition to *P. procumbens* (not included in the present analysis), appear to form a highly homogeneous group, with exclusive synapomorphic characters (such as twin hairs of the cypsela base conspicuously enlarged, and cypsela surface glands positioned between the ribs in the intercostal grooves) and other apomorph-

ic characters that are not exclusive but that are not present in *D. welwitschii* (capitulum made up of "true-ray" marginal florets and actinomorphic disc florets, marginal florets neuter, and cypsela of the marginal florets with rostrum). By contrast, *D. welwitschii* shows a series of apomorphic characters including apex of the disc corolla lobe without thick-bundled veins, disc floret cypsela obconic, and testa of *D. welwitschii* type. The cladogram may thus indicate that *D. welwitschii* (from the central plateaus of Angola, and also present in Zaire) is a distinct genus. Nevertheless, this possibility would have to be confirmed by an analysis including all, or nearly all, species of *Dicoma*.

Of the previously proposed sections of the genus *Dicoma*, *Dimorphae* and *Barbellatae* of Wilson (1923) are clearly paraphyletic (see Table 1, Figs. 10, 11). Considering the sections accepted by Hoffmann (1893a), the results of the present analysis are more or less consistent with *Brachyachaenium* Baker (*D. nachtigalii*), *Macledium* (Cass.) DC. (*D. spinosa* and *D. relhanioides*), and *Pterocoma* DC. (sect. *Plumosae* Wilson) (*D. sessiliflora*, *D. zeyheri*, and *D. saligna*).

Other sections, such as *Steirocoma* DC. (*D. capensis* and *D. niccolifera*, but not *D. elegans*) and *Rhigiothamnus* (Less.) DC. (*D. picta* and *D. fruticosa*), may be monophyletic; however, and apart from the monotypic sections *Hochstetteria* (DC.) O. Hoffm. (*D. schimperi*) and *Eudicoma* DC. (*D. tomentosa*), the species of my *D. tomentosa* group do not appear to form coherent monophyletic groups assignable to any of the sections previously described.

Broadly speaking, the results of the present analysis (Figs. 10–12) suggest that taxa with primitive characters, like those of the *D. carbonaria* group, may be precursors of the genus *Dicoma* that have survived as relict populations in Madagascar. It seems reasonable to hypothesize that these precursors gave rise to a lineage of xeromorphic taxa with small, spiny, coriaceous leaves (such as *D. spinosa*, *D. relhanioides*, *D. grandidieri*, and *D. cana*). The remaining species of the *D. sessiliflora* group, with scarcely coriaceous, scarcely spiny, and more or less broad leaves (*D. sessiliflora*, *D. saligna*, and *D. zeyheri*) probably originated from that lineage in adaptation to moister, shadier conditions. The origin of this group, and of the genus as a whole, would thus appear to have been in Madagascar and southern Africa, as found by Eldenäs and Andenberg (1996) for the genus *Anisopappus* (which has a similar distribution and ecology to *Dicoma*). On this hypothesis, the remaining species of the genus arose, probably later, generally in open dry, including sub-desert and desert, environments.

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THE EVOLUTION OF NON-CODING CHLOROPLAST DNA AND ITS APPLICATION IN PLANT SYSTEMATICS[1]

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ABSTRACT

This article reviews several proposed mechanisms of molecular evolution operating in non-coding regions of the chloroplast genome and argues that awareness and identification of these mechanisms are essential for improving alignment and phylogenetic analysis of non-coding sequence data. The mechanisms are of five categories: (1) slipped-strand mispairing; (2) insertions and deletions linked with secondary structure formations; (3) inversions associated with hairpins and stem-loop structures; (4) localized or extra-regional intramolecular recombination; and (5) nucleotide substitutions. These mutations seem to be largely a function of sequence structure and pattern and may be highly homoplasious in a parsimony topology; therefore, mutations in non-coding regions of the chloroplast genome are described here as structured, nonrandom, and non-independent events. Established methodologies are based in large part on a collective understanding of genic DNA evolution and may need modification when applied to non-coding sequence data. Here I suggest an approach to the phylogenetic study of non-coding cpDNA that incorporates identification of mutational mechanisms in alignment and homology assessment of indels. I also discuss repercussions of non-coding sequence evolution for such aspects of phylogeny estimation as maximum likelihood, distance, and parsimony analysis, the inclusion of indels as phylogenetic characters, and bootstrapping, jackknifing, and "decay" analysis as measures of clade support.

Key words: alignment, intergenic spacers, introns, molecular evolution, mutational biases, mutational mechanisms, phylogenetic analysis, secondary structure.

There is growing interest in comparative analysis of non-coding chloroplast (non-coding cpDNA) sequences for plant systematic studies at low taxonomic levels. Recognition of the limitations of coding (genic) DNA for resolving relationships at these levels inspired the probing of chloroplast introns and intergenic spacers for phylogenetic utility. Underlying this effort was the reasonable premise that non-coding regions experience limited or no selective pressure and are likely to evolve at rates far surpassing those of genic regions (e.g., Curtis & Clegg, 1984; Wolfe et al., 1987; Palmer, 1987, 1991; Olmstead & Palmer, 1994; Böhle et al., 1994). There was also an expectation that non-coding regions should experience random and independent mutations, both in mode and distribution.

For these reasons, a remarkable number of plant systematics studies currently in progress include a molecular component of comparative analysis of non-coding cpDNA sequences. A considerable amount of work already published has demonstrated the potential phylogenetic utility of discrete non-coding regions in the chloroplast: the *trn*L-*trn*F spacer (e.g., Gielly & Taberlet, 1994; Mes & t'Hart, 1994; van Ham et al., 1994; Sang et al., 1997; Cros et al., 1998; Bayer & Starr, 1998), the *trn*T-*trn*L spacer (Böhle et al., 1994, 1997; Small et al., 1998), the *rpo*A-*pet*D and *rps*11-*rpo*A spacers (Peterson & Seberg, 1997), the *atp*B-*rbc*L spacer (Golenberg et al., 1993; Hodges & Arnold, 1994; Natali et al., 1995; Samuel et al., 1997; Savolainen et al., 1997; Setoguchi et al., 1997; Hoot & Douglas, 1998), the *rbc*L-*psa*I spacer (Morton & Clegg, 1993), the *psb*A-*trn*H spacer (Aldrich et al., 1988; Sang et al., 1997), the *acc*D-*psa*I spacer (Small et al., 1998), the *rpl*16-*rpl*14 and *rps*8-*rpl*14 spacers (Wolfson et al., 1991), the intron surrounding *mat*K (Johnson & Soltis, 1994), the *rpo*C1 intron (Downie et al., 1996a, 1996b; Asmussen & Liston, 1998; Downie et al., 1998), the *rpl*16 intron (Jordan et al., 1996; Kelchner, 1996; Kelchner & Clark, 1997; Schnabel & Wendel, 1998; Baum et al., 1998; Small et al., 1998), the *trn*L intron (Sang et al., 1997; Bayer & Starr, 1998; Kajita et al., 1998; Bay-

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er et al., 2000), the *rps*16 intron (Liden et al., 1997; Oxelman et al., 1997), and the *ndh*A intron (Small et al., 1998).

The literature above not only reveals profound differences between the evolution of non-genic and genic cpDNA, but critically contradicts initial assumptions of constraint-free evolution in non-coding regions. Recurring difficulties associated with non-coding sequence data include alternative alignment possibilities of insertions and deletions (indels), regions of length mutation in which homology assessment is questionable or impossible, and the occurrence of localized "hot spots" of inferred excessive mutation, frequently to the point of saturation and loss of phylogenetic signal. How best to proceed with the phylogenetic analysis of such regions should be a topic of considerable concern (see Golenberg et al., 1993; Downie et al., 1996a; Kelchner & Clark, 1997; Sang et al., 1997; Downie et al., 1998).

It is now evident that sequence evolution in non-coding regions of the chloroplast is far more complex than previously supposed. Both introns and intergenic spacers are thought to embody a considerable degree of sequence structure, sometimes in a manner similar to that of ribosomal DNA (rDNA). This structure may generate either regionalized sequence conservation or mutational hot spots of both nucleotide substitutions and insertion/deletion events. Sequence-directed initiators of mutational events may persist as "mutational triggers" (Kelchner, 1996; Kelchner & Clark, 1997), dramatically increasing the possibility of reversal or parallel gain of mutations, particularly length mutations or minute inversions. Hence, there exist essential violations of the assumptions of randomized and independent character evolution embedded in much of the current phylogenetic methodology for comparative sequence analysis—methodology that is based largely on observational comparative study of coding sequence data. Considering that these are today's commonly employed tools for phylogeny estimation based on DNA sequences, there has been as yet remarkably little controversy in the literature about their application to non-genic sequence data.

There are ways to account for mutational patterns observed in non-coding DNA. Comparative studies of non-coding cpDNA sequences during the past decade in particular (e.g., Palmer, 1985; Blasko et al., 1988; vom Stein & Hatchel, 1988; Wolfson et al., 1991; Golenberg et al., 1993; Gielly & Taberlet, 1994; Morton, 1995a; Downie et al., 1996a; Kelchner & Wendel, 1996; Kelchner & Clark, 1997; Sang et al., 1997) have allowed inference of specific underlying mutational mechanisms responsible for generating sequence diversity in non-coding regions of the chloroplast genome. Unfortunately, these mechanisms are often invoked, but rarely incorporated, into the analysis.

Recognition of the potential of structured molecular evolution in non-coding cpDNA regions to improve alignment and assessment of phylogenetic relationships is, I believe, critical for the development of functional molecular systematic research based on non-coding sequence data. Toward this end, I endeavor here to illustrate the following: (1) non-coding regions are highly structured and their elements evolve non-randomly and non-independently; (2) this structure may be used to align the sequence matrix and better assess homology; (3) the resulting gaps in the aligned matrix may contain phylogenetically important information and should be used in a phylogenetic analysis; and (4) the mode of non-coding sequence evolution described here may have potentially serious repercussions for the accuracy of genetic-distance, maximum likelihood, and parsimony analyses, and for bootstrapping and jackknifing techniques. A description of proposed mechanisms of non-coding sequence evolution is followed by a discussion of the appropriateness of current alignment and analysis procedures, with the expectation that it may provide a more informed approach to the application of non-coding sequence data in plant systematics research.

This article is not intended to be a complete review of literature pertaining to the evolution of introns and intergenic spacers in all genomes of an organism. Instead, it serves as a brief review of current literature on non-coding cpDNA regions, and summarizes mutational mechanisms suggested to occur in these regions. Discussed are some of the serious implications this manner of molecular evolution has for the assumptions underlying models employed today by plant molecular systematists.

Mechanisms of Non-Coding Sequence Evolution

The strength of any phylogenetic estimation rests on the accuracy of character homology assessment. Thus, the molecular systematist strives to maximize character homology by the careful alignment of DNA sequences in a data matrix. Fundamental to any alignment procedure of non-coding cpDNA sequence data should be a familiarity with mutational mechanisms directing molecular evolution in non-coding regions. Recognition of these mechanisms as generators of specific mutations can be a powerful tool for the placement of gaps and for the

assessment of probable homology of insertions and deletions (Kelchner, 1996; Kelchner & Clark, 1997).

SLIPPED-STRAND MISPAIRING (SSM)

A widely reported mechanism of length mutation in non-coding regions of the chloroplast is slipped-strand mispairing (SSM). SSM is thought to be a major, even principal, factor in length mutations within non-coding regions of the chloroplast, mitochondrial, and nuclear genomes (e.g., Levinson & Gutman, 1987; Hancock, 1995; Wolfson et al., 1991; Kelchner & Clark, 1997; Sang et al., 1997). Length mutations are important components of non-coding sequence evolution and have been suggested to occur at least as frequently as base substitutions in some chloroplast non-coding regions (Curtis & Clegg, 1984; Wolfe et al., 1987; Zurawski & Clegg, 1987; Clegg & Zurawski, 1992; Golenberg et al., 1993; Gielly & Taberlet, 1994; Clegg et al., 1994).

Slipped-strand mispairing is thought to proceed by a localized mispairing of single-stranded DNA in regions of sequence repeats, as either a string of mononucleotide repeats or tandemly arranged multibase repeat units (Palmer, 1991; Wolfson et al., 1991; Cummings et al., 1994; Hancock, 1995; reviewed by Levinson & Gutman, 1987). Diagrams of proposed SSM mechanics can be found in Levinson and Gutman (1987) and Wolfson et al. (1991). Because A/T-rich regions of bacterial genomes are particularly susceptible to slipped-strand mispairing (Levinson & Gutman, 1987), one could expect a similar effect in the A/T-rich non-coding regions of the chloroplast genome (Wolfson et al., 1991). This is not to imply that SSM acts uniquely on A and T nucleotides; aligned non-coding sequence matrices often infer inserted repeats containing G and C nucleotides, sometimes as pure strings of G or C mononucleotide repeats.

Strings of mononucleotide repeats, particularly of A or T, appear frequently in non-coding cpDNA, and slipped-strand mispairing may potentially generate length mutations within these strings. The difficulty in assessing homology of length variation in long strings of repeats, whether mononucleotide or multinucleotide repeats, derives from the increasing potential for further length mutation relative to string length (Streisinger & Owen, 1985; Golenberg et al., 1993; Kelchner & Clark, 1997; Sang et al., 1997). Subsequent SSM activity may either generate additional repeats of the initial sequence or delete sequence susceptible to slipped-strand mispairing. Perhaps an equilibrium might exist between the probability of inserting subsequent length mutations and the probability of removing sequence from the repeat string. Whether such an equilibrium is present or not, there may be a competitive phenomenon that keeps the length of tandem repeated sequence units continually in flux. Representation of long repeat strings in non-coding sequence alignments would therefore be a "snapshot" of sequences experiencing continual insertions and deletions at that locality.

It follows that a point substitution within a long string of mononucleotide repeat units could act as a stabilizing factor, disrupting its previous uniformity and lowering the probability of further SSM events. Such a substitution would directly influence ensuing mutations in the region and is one example of a non-independent character mutation in non-coding DNA. If the situation were reversed, with a non-homogeneous sequence becoming a string of repeat units, the likelihood of an SSM event would increase and could induce further non-independent mutations by the addition or removal of repeated sequence by slipped-strand mispairing.

As an aid to alignment, SSM-generated insertions and deletions can be used to position and determine number of gaps. A quick study of a repeat unit or the flanking sequence of a gap may be enough to determine if slipped-strand mispairing is the likely progenitor of an observed length mutation. Occasionally, evidence of an SSM event may not be apparent, particularly if a deleted sequence is not a direct repeat of its flanking sequence, or if a subsequent length mutation due to another mechanism obscures an earlier SSM event (Kelchner, 1996).

STEM-LOOP SECONDARY STRUCTURE

Striking to both intergenic spacers and introns in the chloroplast genome is the presence and number of probable secondary structures referred to as "stem-loops." Stem-loops are believed to occur during single-stranding events when inverted repeats meet to form a region of pairing (the stem) surmounted by their interceding sequence (the loop). Such structures have been widely discussed for ribosomal DNA, with ITS and 18S rDNA regions being of particular interest to the plant systematist (see Baldwin et al. (1995), Soltis et al. (1997), and Soltis & Soltis (1998) for discussion of secondary structures in these regions and their phylogenetic implications).

Probable stem-loop secondary structure is commonly reported in non-coding regions of organellar genomes (e.g., Michel et al., 1989; Buroker et al.,

1990; Golenberg et al., 1993; van Ham et al., 1994; Gielly & Taberlet, 1994; Natali et al., 1995; Rigaa et al., 1995; Downie et al., 1996b; Kelchner & Wendel, 1996; Kelchner & Clark, 1997; Sang et al., 1997; Downie et al., 1998). Gielly and Taberlet (1994) reported several probable stem-loops in the *trn*L-*trn*F region of the chloroplast genome, including nine highly probable structures within the *trn*L intron itself. All other introns in the chloroplast genomes of land plants are classified as Group II introns and share a diagnostic secondary structure of six well-defined stem-loop domains (Kohchi et al., 1988; Michel et al., 1989; Downie et al., 1996b; Downie et al., 1998). Diagrams of putative single-stranded secondary structure of introns may be found in Michel and Dujon (1983), Michel et al. (1989), and Downie et al. (1998).

Loop regions of stem-loop secondary structures are often associated with hot spots for mutation in non-coding regions, both of nucleotide substitutions and indel events (vom Stein & Hatchel, 1988; Aldrich et al., 1988; Golenberg et al., 1993; Gielly & Taberlet, 1994; van Ham et al., 1994; Clegg et al., 1994; Ferris et al., 1995; Downie et al., 1996b; Kelchner & Clark, 1997). Indels located in probable loop sequence are frequently inserted or deleted repeat units likely the result of SSM. However, length mutations not attributable to slipped-strand mispairing often occur within loop sequences as well and may be remnants of recombination events.

Although indels are most common in the terminal loop, they may occur anywhere along a secondary structure. For example, Kelchner and Clark (1997) detected what appeared to be an entire deletion of a small sub-loop positioned partway up the stem of an *rpl*16 intron stem-loop in *Oryza sativa*. Such side loops, when present, may be removed in some taxa without compromising the favorability of a stem formation. Occasionally, small segments of the stem itself will be deleted, decreasing the stem length, though perhaps not to an extent that would annihilate possible secondary structure formation.

Very large loops are often associated with regions of chaotic or "labile" length variation characteristic of many non-coding cpDNA sequence matrices (e.g., Golenberg et al., 1993; Downie et al., 1996a; Soltis et al., 1996; Kelchner & Clark, 1997; Baum et al., 1998). Homology assessment here can be difficult or impossible, and the conservative approach of removing these regions from the data matrix before phylogenetic analysis is frequently adopted.

In contrast to the loop of stem-loop secondary structures being highly susceptible to nucleotide substitutions and length mutation, the inverted repeated sequence composing the stem is frequently conserved in character (Learn et al., 1992; Gielly & Taberlet, 1994; Downie et al., 1996a, 1996b; Kelchner & Clark, 1997), particularly when stems are long and possess highly favorable energy of formation values (ΔG values; see Kelchner & Wendel, 1996; Dumolin-Lapègue et al., 1998). A sequence involved in stem formation is less available for substitution and length mutation because it is paired with its sister repeat; this can engender non-randomly and non-independently evolving sequence units.

Similar to ribosomal RNA and rDNA secondary structure (e.g., Curtiss & Vournakis, 1984; Wheeler & Honeycutt, 1988; Dixon & Hillis, 1993; Soltis & Soltis, 1998), a nucleotide substitution occurring in a stem sequence of a non-coding cpDNA region could compromise secondary structure formation. Compensatory mutation may then occur to preserve the potential for structure formation (Kelchner, 1996; Kelchner & Clark, 1997). Although sequence conservation may be present merely as a function of sequence pattern (perhaps the case in intergenic spacers), the degree of secondary structure conservation in a chloroplast Group II intron suggests secondary structures are integral to proper functioning of the intron (Clegg et al., 1986; Learn et al., 1992; Downie et al., 1996a). Experimental evidence has shown some of this structure is essential for auto-splicing mechanisms in Group I and II introns (Bonnard et al., 1984; Kohchi et al., 1988; Dujon, 1989; Cech, 1990; Michel & Westhof, 1990; Hibbett, 1996).

Identification of probable secondary structure can be valuable when aligning and analyzing non-coding sequences by improving gap positioning and the appraisal of character homology. Gaps flanked by inverted repeats and regions relatively rich in G and C content are suspect as possible stems of secondary structures. As noted, regions of chaotic length mutations are correlated with loops, so the boundaries of a chaotic region will frequently correspond with inverted repeats that can form a stem, even if they do not directly neighbor the chaotic region. Computer programs such as OLIGO (Rychlik & Rhoads, 1989), MULFOLD (Jaeger et al., 1989; Zuker, 1989), and GCG's Stemloop (Genetics Computer Group, Madison, Wisconsin) can assist in the detection of secondary structure in non-coding sequences. A search can be conducted by hand, particularly if a published data set exists for the region. Free energy of formation values (ΔG) can be calculated with some of the prior software as an appraisal of the likelihood of formation of a partic-

ular secondary structure (see Kelchner & Wendel (1996) for an example where ΔG values were applied to parallel inversion events in their data).

MINUTE INVERSIONS

Minute inversions of four to six base pairs have been linked to small stem-loop secondary structures commonly referred to as hairpins (Kelchner & Wendel, 1996). Hairpins consist of a stem composed of nearly adjacent inverted repeats producing a stem-loop structure with a particularly small loop. This loop may become inverted by recombination, and the inversion may be so small that it either escapes notice during alignment (Kelchner & Wendel, 1996; Kelchner & Clark, 1997), or the inverted sequence matches particular bases of the uninverted sequence, resulting in a confusing array of minute gaps (see Golenberg et al., 1993).

Identifying minute inversions can require careful attention when aligning sequence data, particularly if alternative gap weighting schemes of an alignment program have not been rigorously explored. Candidates for a hidden inversion are several adjacent nucleotide substitutions, a series of tiny gaps, or a gap that demonstrates no repeat aspect to its sequence structure. Alternatively, one could investigate these probable secondary structures by hand or with a secondary structure computer program. Failure to recognize minute inversions in a sequence data set has several repercussions for phylogenetic analysis, discussed fully in Kelchner and Wendel (1996) and summarized here in Analysis of Non-Coding Sequence Data.

Finally, small inversions associated with hairpins may be highly susceptible to reversal and parallelism within a study group, even at the interspecific level (Kelchner & Wendel, 1996; Kelchner & Clark, 1997; Sang et al., 1997; Dumolin-Lapègue et al., 1998). This susceptibility to reversal or parallelism is due to the persistence of the mutational trigger (Kelchner & Clark, 1997)—the nearly adjacent inverted repeats—after the initial inversion event.

NUCLEOTIDE SUBSTITUTIONS

Nucleotide substitutions are generally reported as being more common in non-coding than in coding regions (Wolfe et al., 1987; Zurawski & Clegg, 1987; Olmstead & Palmer, 1994; Hoot & Douglas, 1998; however, see Sang et al., 1997, for an exception). Surprisingly, a number of studies report nucleotide substitutions as being just equal to or less frequent than length mutations in closely related taxonomic groups (Curtis & Clegg, 1984; Wolfe at al., 1987; Zurawski & Clegg, 1987; Clegg & Zurawski, 1992; Golenberg et al., 1993; Gielly & Taberlet, 1994; however, see Small et al., 1998).

Percent AT content is quite variable in non-coding cpDNA regions, though it is generally higher than the average value for the chloroplast genome (Shimada & Sugiura, 1991; Downie at al., 1996a; Small et al., 1998). Because of their high AT content, non-genic regions must make a significant contribution to the high overall frequency of A and T in the chloroplast genome. Kajita et al. (1998) reported an AT content of 67% in the *trn*L-*trn*F spacer and *trn*L intron, Kelchner and Clark (1997) reported 70.5% AT composition in the intron of chloroplast gene *rpl*16 in bamboos, and Small et al. (1998) found an incredible 77.1% AT content in the intergenic spacer *trn*T-*trn*L in *Gossypium*. Undoubtedly, this unequal tendency toward AT richness in non-genic chloroplast DNA has several as yet undetermined implications for phylogenetic analysis of non-coding sequence data. At a minimum, it introduces a strong base composition bias into the analysis.

Substitutions may demonstrate rather high levels of homoplasy in non-coding cpDNA regions due to the frequency of inferred multiple-hit sites (nucleotide sites experiencing multiple substitution events). Multiple-hit sites occur even at very low estimates of percent sequence divergence (Kelchner, 1996; Kelchner & Clark, 1997), suggesting that the accepted coding region estimates of "around 10–15%" sequence divergence for optimal phylogenetic signal may be inadequate measures for phylogenetic utility of a non-coding region.

Precise understanding of mechanisms underlying multiple-hit substitutions in non-coding DNA is lacking. However, attributes of the molecular evolution of non-coding regions influence the manner of nucleotide mutation or the distribution of nucleotide substitution events in an intron or intergenic spacer. Stem sequence and loop regions may differentially permit mutations, resulting in non-randomly distributed and non-independent nucleotide substitutions. Statistical significance of differential mutation rates in loops relative to stems may be tested for an adequate distribution model (see Olmstead et al.'s (1998) test for stochastic mutation in the chloroplast genes *ndh*F and *rbc*L), yet has rarely, if ever, been performed on non-coding cpDNA data sets.

In addition to secondary structure affecting the random distribution of nucleotide substitutions, there may be constraints on the type of mutation an individual site experiences. For example, there is a correlation between transition/transversion ra-

tios and neighboring base composition in non-coding regions (Morton, 1995a, b; Morton et al., 1997; Savolainen et al., 1997). The correlation suggests that nucleotides flanked by A and/or T will demonstrate a significant tendency toward transversion mutations. Such a tendency limits possible nucleotide replacements at these sites, increasing the chance of parallelism and reversals, particularly if the site experiences multiple hits. One would also expect transversion substitutions to be more common in data sets of high AT content.

INTRAMOLECULAR RECOMBINATION

Intramolecular recombination on an extra-regional or genomic scale has been suggested between adjacent or nearby repeats in the chloroplast genome (Howe, 1985; Palmer et al., 1985; Palmer et al., 1987; Blasko et al., 1988; Ogihara et al., 1988; Milligan et al., 1989; Kanno & Hirai, 1992; Kanno et al., 1993; Morton & Clegg, 1993; Hoot & Palmer, 1994). In the context of non-coding sequence comparison, such a large-scale recombination involving the particular region of study could result in indels of surprising size that contain sequence content not readily identifiable in origin.

Recombination events may operate on a finer scale within a discrete non-coding region. Occasionally one infers extensive deleted sequence in an alignment with no apparent mechanistic explanation, presence of a small or moderately sized inversion, or a large insertion showing little congruence with surrounding sequence pattern. Such mutations suggest intramolecular recombination, and they frequently occur in the loop regions of probable secondary structures. Sequences involved in stem-loops may be particularly susceptible to recombination events due to the conserved inverted repeats and mutationally flexible loop. Therefore, such structures could experience interactive recombination with other stem-loops, particularly with those existing in complementary sequence position.

Recombination involving the entire loop of a secondary structure may occur, particularly in structures with long stems, resulting in minute or moderate-sized inversions in both intron and intergenic spacer regions (Natali et al., 1995; Kelchner & Wendel, 1996; Kelchner & Clark, 1997; Sang et al., 1997). Such incidents are often homoplasious (Kelchner & Wendel, 1996; Kelchner & Clark, 1997; Sang et al., 1997; Dumolin-Lapègue et al., 1998) due to the persistence of the mutational trigger; in this case, the hairpin stem.

Intramolecular recombination is a notable alternative to slipped-strand mispairing as a source for certain inserted or deleted tandem-repeat length mutations (Palmer, 1985; Blasko et al., 1988). However, Wolfson et al. (1991), Sang et al. (1997), and Kelchner and Clark (1997) suggested SSM is a more likely mechanism for length mutation in their studies of chloroplast introns and intergenic spacers.

ALIGNMENT

There are many philosophies for sequence alignment, and much of the literature centers on the proper application of computer software for this purpose. The structure present in a non-coding cpDNA sequence makes it an excellent example for discussing what I believe to be the fundamental problem of most computer alignment programs: defining the nucleotide as a discrete and independent character. The identification of secondary structure and mutational mechanisms in the data may greatly improve on current algorithmic alignments of gaps, and thus on assessment of character homology.

Many have found software, particularly versions of CLUSTAL (Higgins et al., 1992; Thompson et al., 1994), to be of help at least initially with the alignment of non-coding sequences. The alignment is then subjected to an "improvement by hand" to position gaps (e.g., Samuel et al., 1997; Downie et al., 1998; Bayer & Starr, 1998; Kajita et al., 1998). This procedure saves time if the sequences are similar in length, but when indels become numerous in the data matrix the difficulties of alignment dramatically increase. This is because most alignment software initially regards each character in the matrix as an independent unit, unless otherwise specified by particular position or gap weighting schemes defined by the user. The software is incapable of determining when mutations other than substitutions have arisen, such as non-independent insertions, deletions, or inversions correlated with SSM and secondary structure. Appropriate weighting for these mutations that could be incorporated into an alignment algorithm is, at present, undeveloped.

The Elision method of Wheeler et al. (1995) attempts to improve gap placement and indel homology by alignment software. The Elision method uses standard alignment algorithms to produce a series of competing alignments based on varying gap weighting schemes. These competing alignments are then combined in a single matrix and an analysis is performed, with the effect that support is increased for aligned regions that most frequently appear among the various gap-weighting schemes. This method aims at objectivity, but makes no im-

provement on the alignment algorithm's inability to assess mutation types other than independent point substitutions. Mutations in non-coding regions are influenced by surrounding sequence structure and frequently occur not as independent base mutations but as linked multinucleotide mutation events, like the insertion of a repeat unit (Kelchner, 1996; Kelchner & Clark, 1997). The likelihood that many non-coding mutations are derived from sequence fragments that are inserted, deleted, inverted, or otherwise rearranged, negates the assumption of discrete, independent nucleotide characters underlying all alignment algorithms, as well as any extension of those algorithms like the Elision method.

At a minimum, those using sequence alignment programs to establish putative homology of characters in their data matrix should experiment with a wide variety of gap-weighting options. These options, however, may not reveal the underlying mutational mechanisms occasioning sequence rearrangements in chloroplast non-coding regions. They may, however, facilitate the rapid alignment of segments of the matrix that share consistent sequence integrity and thus pinpoint regions of variable length that require special consideration.

Alternatively, some have avoided alignment programs entirely and describe aligning sequences by hand (e.g., Golenberg et al., 1993; Hodges & Arnold, 1994; Kelchner & Clark, 1997). This approach facilitates a careful study of the matrix as it forms and increases the researcher's familiarity with mutations in the sequences. However, alignment by hand, especially when dealing with considerably divergent taxa or with the presence of a great number of length mutations, can be tedious and time consuming.

Kelchner and Clark (1997) suggested that awareness of the proposed mutational mechanisms active in non-coding regions can be useful for inferring and positioning gaps and ultimately in assessing homology. Golenberg et al. (1993) were the first to detail a criterion for aligning gaps in non-coding cpDNA matrices. Based on their example, Kelchner (1996) and Kelchner and Clark (1997) modified the alignment criterion for chloroplast *rpl*16 intron sequences. Hoot and Douglas (1998) also revised Golenberg et al.'s (1993) method of gap alignment, framing the beginnings of a nomenclatural procedure for defining gap categories. Although a nomenclatural system is not requisite for gap treatment in a phylogenetic analysis, it may be useful in collating information of inferred mutational mechanisms if universally applied in non-coding DNA studies.

ALIGNMENT ISSUES: EXAMPLES FROM NON-CODING cpDNA DATA

Here I present examples (Kelchner & Wendel, 1996; Kelchner & Clark, 1997; Kelchner, unpublished data) to illustrate the inference of mutational mechanisms in non-coding cpDNA sequences and demonstrate the practice of applying mechanistic explanations to alignment and homology assessment. Nucleotides in lower-case bold print are inferred insertions; underlined nucleotides indicate the probable progenitor sequence of an insertion or, in Examples 4 and 5, call attention to a particular sequence of interest.

A common type of insertion in non-coding cpDNA is a direct repeat of a neighboring sequence ("Type 1a" gap; Golenberg et al., 1993; Hoot & Douglas, 1998). These often take the form of variable-length strings of a mononucleotide repeat unit (Example 1).

EXAMPLE 1.

1. TTAAAAAAAAA---TTGA
2. TTAAAAAAAAAA--TTGA
3. TTAAAAAAAA----TTGA
4. TTAAAAAAAAAAAATTGA

Homology can be highly uncertain for these repeated nucleotides. Therefore, such regions are either removed from consideration as potential phylogenetic characters (a conservative approach) or included as coded gap characters corresponding to length of the repeat string (often becoming highly homoplasious in the context of a resulting topology). Uncertainty of homology is exacerbated by potential inaccuracies of enzymatic processes during PCR amplification and sequencing, which can also generate variable-length repeat strings independent of the template's sequence constitution. When strings of adjacent mononucleotide repeats are highly variable in length in a matrix and reach or exceed the range demonstrated above, they become more likely to experience further SSM mutation. For this reason, it is perhaps most reasonable to remove such areas from consideration in a phylogenetic analysis.

Insertions can also be multinucleotide repeat units of a neighboring sequence, as demonstrated in Example 2 by the inserted repeat unit **ataaa** ("Type 1b" gap; Golenberg et al., 1993; Hoot & Douglas, 1998).

EXAMPLE 2.

1. ATAAAACAAA-----GAGCG
2. ATAAAATAAA**ataaa**GAGCG
3. ATAAAATAAA-----GAGCG
4. ATAAAATAAA-----GAGCG

An inserted repeat of this nature could be extensive in length and may be difficult to recognize as a repeat unit during alignment (for example, I have identified a 73 bp inserted repeat [unpublished data] in the *trn*T-*trn*L intergenic spacer in Myoporaceae). A repeat unit by its very nature shares nucleotide content and order with flanking sequence; therefore, multiple gaps may be inferred by pairing segments of an inserted repeat with its progenitor sequence. This is particularly problematic if the insertion or its progenitor has experienced subsequent nucleotide substitutions.

Even when a single gap is inferred, positioning of the gap may hide evidence that the insertion is a repeat unit. Example 3 is reproduced from Kelchner and Clark (1997) and demonstrates how a repeat unit may be obscured in a sequence matrix.

EXAMPLE 3.

A.

1. GGTTATGA ----- ATTAACA
2. GGTTATAA ----- ATTAACA
3. GGTTATAA **tataa** ATTAACA
4. GGTTATAA **tataa** ATTAACA

B.

1. GGTTAT-- ---GA ATTAACA
2. GGTTAT-- ---AA ATTAACA
3. GGTTATAA **tataa** ATTAACA
4. GGTTATAA **tataa** ATTAACA

C.

1. GGTTA--- --TGA ATTAACA
2. GGTTA--- --TAA ATTAACA
3. GGTTATAA **tataa** ATTAACA
4. GGTTATAA **tataa** ATTAACA

Alignment possibilities A, B, and C were equally probable using CLUSTAL W (Thompson et al., 1994). Only alignment A reveals the insertion is a repeat unit—a common mutation type in non-coding regions. If alignment options B or C were used for phylogenetic analysis, the content of the insertion would be of unexplainable origin (though still possible) and the potential of incorrectly assessing nucleotide homology in the region may be considerable.

Any of the gap positions in this particular example would not affect a topology generated from these four taxa, but gap positioning may have a significant effect in a larger matrix of more distantly related taxa. The position of the gap in alignment 3A and detection of the repeat unit may also be relevant in determining a weighting scheme for these non-independent characters.

Length mutations may overlap with one another to create a progressive-step indel. In the more extreme cases, appraisal of homology in these regions can be very difficult, or impossible (Palmer et al., 1985; Downie et al., 1996b; Kelchner & Clark, 1997). Example 4 demonstrates a probable progressive-step indel in which two possible placements exist for the repeat TTGA. Note that the underlined sequence is a direct repeat of the preceding sequence TCGTAATTGA in the matrix.

EXAMPLE 4.

1. AATCGTAATTGA ---------- ----AACAGA
2. AATCGTAATTGA ---------- ----AACAGA
3. AATCGTAATTGA TCGTAA**TTGA** ----AACAGA
4. AATCGTAATTGA TCGTAA**TTGA** ----AACAGA
5. AATCGTAATTGA TCGTAA**TTGA** **ttga**AACAGA

If part of the underlined **TTGA** in sequences 3 and 4 is moved from its current position to align with **ttga** in sequence 5, the possibility that the **ttga** sequence is a direct repeat of the preceding sequence may be obscured; however, this alignment choice would not be impossible. As the preceding sequence to the underlined 10 bp repeat does not contain this additional **ttga** repeat, we can infer that two separate events have given rise to an initial 10 bp insertion in sequences 3 and 4, followed by an additional 4 bp insertion in sequence 5. Whether **ttga** itself or the preceding **TTGA** is the subsequent inserted mutation is impossible to determine. In this case, either alternative alignment of the TTGA unit would cause no effect in a phylogenetic analysis; it is most important here to discern the two length mutation events. If any potentially informative nucleotide substitutions were present in either of the repeat units in Example 4, these substitutions should be excluded from a phy-

logenetic analysis on the basis that nucleotide homology of the repeats is not discernable.

The example above suggests that homology may be indicated by the length of insertions or deletions in a gap, although such an assumption is not without risk. Example 5 below demonstrates multiple possible alignments of the **gatt** repeat unit (represented individually by sequences 2, 3, and 4) with the insertion in sequence 1.

EXAMPLE 5.

1. CAGATTGATTGATTATTATACTGATTATGC
2. CAGATT----------**gatt**ATGC
3. CAGATT**gatt**----------ATGC
4. CAGATT----**gatt**----------ATGC
5. CAGATT----------ATGC

Again, actual homology is impossible to assess with confidence, for there exist three GATT repeat units in the insertion in sequence 1. In cases like this, homology is often inferred on the basis of length of indel and minimum number of gaps required to position the repeat. Hence, the **gatt** repeats in sequences 2, 3, and 4 would be aligned one above the other and on one side of the gap to reduce the number of inferred indel events. When coding indels as characters, this would be a reasonable solution in lieu of other evidence for indel origin, and the repeat **gatt** would be treated as homologous for those sequences that contain it.

Equal length of insertions may not be strong evidence of their homology (Kelchner, 1996; Kelchner & Clark, 1997; Hoot & Douglas, 1998). Consider the insertions in Example 6A.

EXAMPLE 6A.

1. GGTTAAT **tctat** TCTATCT
2. GGTTAAT **ttaat** TCTATCT
3. GGTTAAT ttaat TCTATCT
4. GGTTAAT ----- TCTATCT
5. GGTTAAT ----- TCTATCT

Alignment of the insertions in Example 6A results in the probably mistaken homology of indels in sequences 2 and 3 with that of sequence 1. The insertion in sequence 1 likely arose from an inserted repeat of the sequence to the right of the gap, TCTAT. This would be a more parsimonious explanation, in terms of total number of mutation events, than to infer a single inserted repeat followed by two adjacent nucleotide substitutions in sequence 1. Sequences 2 and 3 probably share a similar origin as a repeat of the preceding sequence TTAAT. The events, aligned as they are in Example 6A, are probably non-homologous. A re-alignment could be performed to accommodate the two separate indel events (Example 6B), even though the insertions are of the same length and the alignment infers an additional gap (see Hoot & Douglas, 1998).

EXAMPLE 6B.

1. GGTTAAT ----- tctat TCTATCT
2. GGTTAAT ttaat ----- TCTATCT
3. GGTTAAT ttaat ----- TCTATCT
4. GGTTAAT ----- ----- TCTATCT
5. GGTTAAT ----- ----- TCTATCT

There is a hazard that minute inversions (Kelchner & Wendel, 1996) can be completely obscured in a matrix if they introduce no gaps during alignment, particularly if alternative gap-weighting schemes have not been rigorously pursued. If present and unrecognized in a data matrix, minute inversions may overweigh a particular mutation by interpreting the single mutation event (an inversion) as multiple apomorphies of adjacent nucleotide substitutions. Example 7 below illustrates a situation in which sequences 2 and 3 share the inversion **TTGG** to **CCAA** (from Kelchner & Wendel, 1996).

EXAMPLE 7.

1. TAATATT **TTGG** AATATTA
2. TAATATT **CCAA** AATATTA
3. TAATATT **CCAA** AATATTA
4. TAATATT **TTGG** AATATTA
5. TAATATT **TTGG** AATATTA

If the inversion is of sufficient length to introduce multiple gaps in the matrix (see Golenberg et al., 1993; Sang et al., 1997), two possibilities can occur: the gaps will be misaligned to parts of the inverted sequence sharing spurious sequence similarity with the uninverted sequences; or, there will be inference of an inserted sequence of unknown origin (in reality, the inverted nucleotides), which corresponds with a deletion in the homologous uninverted sequences. Each possibility will lead to inaccurate assessment of homology and may potentially have a considerable effect on phylogeny estimation.

Regions in the matrix demonstrating many independent variable-length insertion and deletion

events will likely be associated with secondary structures, specifically with loop regions of stem-loops (Kelchner, 1996; Downie et al., 1996b; Kelchner & Clark, 1997). Identification of flanking sequences involved in possible stem formation could locate the boundaries for the region and aid in aligning the indels. Discerning probable SSM-susceptible sites can also be informative for the inference of parallel and reversed insertions or deletions.

Perhaps methods of gap or character weighting and alignment based on mechanisms of mutation can be incorporated into software designed for non-coding sequence alignment, particularly by including an evaluation of ΔG values for probable secondary structures. However, the diversity of rates and types of molecular evolution in non-coding regions may be profound. As with coding DNA, we are far from understanding all forces directing non-coding molecular evolution to a degree that we can, with any certainty, assign probabilities to individual mutations.

Considering that alignment of sequence data is fundamental to the entire phylogeny estimation process, authors should more fully describe the steps taken to align their sequence data in order to provide necessary information for the assessment of their proposed reconstructions of phylogenies.

Analysis of Non-Coding Sequence Data

The mechanisms of evolution described above have a number of significant implications for the phylogenetic analysis of non-coding sequence data. Among these are the following:

(1) Slipped-strand mispairing can be the result of persistent mutational triggers (especially when the trigger sequence is located in the stem of a stem-loop secondary structure). This can introduce homoplasy from parallelisms and reversals into any phylogenetic estimations that include gap-coded characters in the matrix. Multiple indel events in a localized region may obscure homology of length mutations. Non-independence of these mutations introduces the issue of relative weighting of nucleotide characters linked in a repeat unit, if each base is treated as a character in an analysis. Weight of the unit taken as a single character is also an issue if the unit is included in the analysis as a coded gap character.

(2) Secondary structure shows nonrandom mutation in the form of compensatory mutation and possible homogenization of sequence necessary for stem formation. Loop sequence is available for multiple mutations in the form of inversions, length mutations, and multiple-hit point substitutions, any of which may obscure evolutionary history.

(3) Inversions may show high levels of parallelism and reversal, and their phylogenetic utility may not be particularly robust. Undetected minute inversions may be buried within a data matrix and consequently treated as multiple base substitution synapomorphies instead of a single mutational event.

(4) Nucleotide substitutions may be under peculiar constraints not fully understood. There is evidence of a bias in non-coding regions involving transition/transversion substitution ratios due to the influence of neighboring bases. A particular base may experience substitution events multiple times in closely related lineages, reaching saturation long before the expected saturation level for the remaining sequence. A base-composition bias toward A/T content is clearly present in non-coding cpDNA.

Selective pressures exerted on non-coding regions may be largely a function of the physical structure of the sequence and possible functionality of introns and intergenic spacers. Reliance on methodology developed for coding sequence, which includes estimates of constraints on coding sequence evolution, transition/transversion ratios, and mutation probabilities, is inappropriate for the analysis of non-coding regions.

Phylogenetic estimations based on genetic distance measures of non-coding cpDNA sequences must be approached with care. Superficial application of models for maximum likelihood (ML; Felsenstein, 1981) or neighbor-joining (NJ; Saitou & Nei, 1987) could easily produce erroneous phylogenetic estimations if several key assumptions underlying the methodology are violated.

For example, most models consider a nucleotide site as the unit of evolution (Ritland & Eckenwalder, 1992), a consideration that is contradicted by the mode of non-coding sequence evolution. Simplistic models based on the commonly calculated Kimura estimates (Kimura, 1980) and Jukes-Cantor estimates (Jukes & Cantor, 1969) assume an equal 25% frequency for each nucleotide type throughout the sequence and generate base mutation probabilities from this assumption. Because non-coding cpDNA regions can demonstrate much higher A/T content, this assumption is clearly contradicted. Furthermore, transition/transversion ratios in non-coding regions can differ considerably from coding ones (see Hoot & Douglas, 1998), and may even vary between discrete non-coding regions of the chloroplast genome. Among-site mutation rate heterogeneity is highly probable, especially if regions of conservation and hot spots for mutation exist in

the data. The presence of multiple gaps in an aligned matrix presents an additional hurdle for distance analysis, and indels themselves are difficult to incorporate as additional characters.

Countering such complications can be involved and computationally demanding. Modification of the initial Jukes-Cantor estimates to allow for varying base frequencies (e.g., Tajima & Nei, 1984) should be employed. Transition/transversion ratios can be estimated directly from the non-coding sequence matrix by pairwise sequence comparisons (e.g., Yang & Yoder, 1999), eliminating the circularity occasioned by measures derived from a topology. More refined distance models that incorporate these problems stand a better chance of reflecting the underlying manner of molecular evolution in non-coding sequence data. Such refined models may therefore estimate a more accurate phylogeny that better recovers the evolutionary history of the characters.

With ML, transition/transversion estimates are dependent on whether among-site rate variation has been incorporated in the model and can be sensitive to the accuracy of the topology used for their estimation (Sullivan et al., 1996). Among-site rate heterogeneity in the data is often assumed to fit either a negative binomial or gamma distribution function, and confirmation can be assessed statistically. Such rate heterogeneity is likely present in non-coding sequence data due to the effects of secondary structure on mutation likelihoods. Rates of variation at sites are usually expected to fit a gamma distribution model (Yang, 1996), and a parameter (α) can be determined to define the shape of that underlying function in an ML analysis (see Yang (1994) and Yang (1996) for thorough explanation). However, Sullivan et al. (1996) suggested α estimates are strongly affected by the topology used for their estimation. Therefore, to improve the ability of a model incorporating gamma distribution to recover the "correct" phylogeny, α must be calculated directly from the data matrix; this should be done by pairwise comparison, which can be a computationally intensive or even impossible procedure as the number of taxa increases in the matrix (Yang, 1996; Sullivan et al., 1996). Poor estimation of α can easily result in a misleading phylogenetic hypothesis (Yang, 1996; Sullivan et al., 1996).

Other problems associated with non-coding cpDNA sequence data may be very difficult to address. If at least some of the mutation in non-coding sequences occurs in linked units, then the non-independence of these nucleotide characters directly affects the subsequent analysis. At present, there is no reliable parameter estimate to incorporate such non-independent characters in a distance model. Most work on parameter estimates for models has been based on coding sequence observations, and thus may not reflect the unique aspects of molecular evolution in non-coding regions.

Determining probabilistic estimates for non-coding cpDNA mutations is, at this time, difficult; therefore, the accurate assessment of the underlying mode of evolution for maximum likelihood analysis may be impossible. As Yang et al. (1995) discussed in detail, the accuracy of ML in recovering an evolutionary history is strongly dependent on the evolutionary model applied. Thus, for non-coding cpDNA sequence data (as well as genic sequence data), deeper understanding of the manner of evolution in these regions is required before an accurate model for ML phylogenetic analysis can be applied.

The frequent alternative to distance measures and maximum likelihood is parsimony analysis. Heuristic parsimony searches can be considerably faster and less computationally intensive than a maximum likelihood analysis with the parameter adjustments described above; however, they are often much slower than a distance analysis. Parsimony analyses that contain no weighting schemes for transition/transversion bias and non-independent mutation of matrix characters may be as vulnerable to recovery of an inaccurate phylogeny as similarly simplistic distance models. It has been suggested that parsimony's potential in some cases to recover a correct topology decreases significantly when among-site rate heterogeneity exists in the data (Tateno et al., 1994; Kuhner & Felsenstein, 1994; Huelsenbeck, 1995). Such rate heterogeneity could arise from the structured sequence patterns described here in non-coding cpDNA. And though it has been proposed that the reliability of parsimony estimates increases with increasing number of taxa included in an analysis (e.g., Wakeley, 1993; Sullivan et al., 1995; Yang, 1996), it is unclear if this effect is independent of possible among-site rate variation.

Parsimony specifies no particular probabilistic evolutionary model, but like all phylogenetic estimation methods it is influenced by non-independence of characters. This problem can be alleviated to a degree if mutations such as inversions and inserted or deleted repeats are recognized as non-independent events and are either excluded from the analysis or coded separately as described below. Any non-independent evolution of neighboring nucleotides in a sequence would create an artificial weighting effect for these positions in a parsimony

analysis that considers each nucleotide an independently evolving character.

Various weighting schemes have been proposed to counter this effect. Weighting has been applied, for example, to compensatory mutations associated with secondary structure in rDNA (e.g., Wheeler & Honeycutt, 1988; Dixon & Hillis, 1993; Baldwin et al., 1995; Soltis et al., 1997; Soltis & Soltis, 1998). Trial weighting schemes have also been applied to non-coding sequence data from the chloroplast (e.g., Downie et al., 1996a; Liden et al., 1997). However, Olmstead et al. (1998) reasoned that an erroneous weighting model increases the chance that the correct topology is excluded from the most parsimonious topologies recovered. In their opinion, a more general model such as equal weighting of characters may limit resolution, but would increase the chance that the "true" tree is recovered by the analysis. Development of defensible weighting schemes for non-coding sequence data would necessarily come from evidence provided by comparative analysis of non-coding regions throughout the chloroplast genome, and may be specific to individual data sets. The likelihood of misdiagnosing an appropriate weighting scheme for subsets of the data may still be high. Therefore, it is perhaps sensible for now to apply equal weighting to non-coding sequence characters until we have further evidence to support a particular weighting scheme.

Insertions and deletions have been shown to be of considerable phylogenetic value (e.g., Golenberg et al., 1993; Mes & Hart, 1994; Natali et al., 1995; Downie et al., 1996a; Kelchner & Clark, 1997; Oxelman et al., 1997; Sang et al., 1997; Liden et al., 1997; Downie et al., 1998; Bayer & Starr, 1998), and one should consider including gaps as coded (present/absent) characters appended to the sequence matrix (e.g., Hodges & Arnold, 1994; Kelchner & Clark, 1997; Sang et al., 1997; Downie et al., 1998; Hoot & Douglas, 1998; Bayer & Starr, 1998). Selection of gaps to be included in the analysis, however, is somewhat subjective in that optimally only those length mutations arguably homologous based on size, composition, and related mechanistic origin should be included.

The exclusion of gaps and removal of coded gap characters from a non-coding sequence matrix can be an interesting and informative approach to studying the degree of resolution provided by point substitution information alone (e.g., Kelchner, 1996; Kelchner & Clark, 1997). A similar analysis can be conducted by including coded gap characters only and excluding all other characters in the matrix. Coupled with mapping characters onto a topology produced from a complete matrix, these partitioned analyses may prove useful in locating and determining the degree of problematic homoplasy affecting resolution in competing topologies.

Minute inversions should be identified and removed from the analysis, to be added as present/absent characters at the end of the matrix (Kelchner & Wendel, 1996; Kelchner & Clark, 1997). This eliminates potential scoring of multiple non-homologous synapomorphies that are artifacts of an inversion mutation.

Of some concern is the tendency to treat nucleotide gap characters of taxa that do not share an insertion (i.e., have only spaces present at the insertion position in the matrix) as missing characters when conducting parsimony analysis. This results in inferred nucleotide homology for characters in the inserted sequences, which leads to cladistic assessment of their base substitutions. Such an approach should be applied only when evidence of the homology of inserted sequences is convincing. Chaotic regions or other areas where homology assessment is deemed impossible should be excluded from the data matrix before analysis (see Liden et al., 1997) to avoid this mistaken claim of nucleotide homology.

Bootstrap (Felsenstein, 1985) and jackknife (Farris et al., 1997) analyses, frequently misunderstood to be direct measures of phylogenetic accuracy, are only as sound as their underlying analysis procedure. As with coding sequences (see Trueman, 1993; Hillis & Bull, 1993; Bremer, 1994; Mishler, 1994; Brown, 1994), both support measures can be affected by the non-independent structure present in non-coding sequences. The structure invalidates a requirement of the statistic that each nucleotide be a discrete and independent character.

Bootstrap and jackknife analyses are a re-sampling of the data matrix in an effort to statistically measure how robustly the data in the matrix support a particular topology. The concept is sound, but the statistical integrity of both measures relies on the assumption that each nucleotide is an individual character, that each character evolves randomly and independently, and that the matrix represents a sample of a much larger population of characters evolving in identical fashion (Felsenstein, 1985). Due to the non-independent structure existing in non-coding regions, and the probably unique series of evolutionary constraints acting not only on individual non-coding regions but also on partitions of a region, each of these assumptions may be violated. Sampling from within such a data set equates to sampling a nonrandom and non-independent subset of a non-existing larger population. A large number of bootstrap replicates should,

in theory, cover all possible error due to reduced character sampling in each replicate, but the strength of the bootstrap test is weakened if the characters are not accurately defined. If a character in some cases is not an individual nucleotide but a suite of nucleotides, the conditions that would make bootstrapping and jackknifing accurate as measures of data support for a topology are not satisfied. An analysis would produce an unequal weighting effect on subsets of the data in each resampling due to the frequent localized violation of character definition.

A non-resampling technique that allows assessment of data support for individual clades is the Bremer Support measure (BS, or "decay" analysis; Bremer, 1988, 1994; Donoghue et al., 1992; for application to large data sets, see Baum et al., 1994; Morgan, 1997). The measure is a function only of the recoverability of clades in topologies progressively one step longer. Bremer support has the possibility of sidestepping the effects of character definition issues discussed above for bootstrapping if the model underlying the phylogeny estimation considers the variable nature of character definition in a nucleotide set.

Oxelman et al. (1999) demonstrated that bootstrapping and BS evaluate different parameters of the data matrix, and are thus not directly comparable measures (though BS values, when high, may be imperfectly correlated with bootstrap and jackknife values). BS values cannot be viewed as probabilistic estimates themselves (Oxelman et al., 1999), and an inability to adapt the measures to a standard scale that is universally applicable renders the technique of dubious worth to some systematists. However, the innovation by Oxelman et al. (1999) that includes minimal branch length values with each BS value does, in a non-standard way, improve the comparative information capacity of the measure. This procedure may be more meaningful and informative than bootstrap and jackknife values for non-coding cpDNA data.

Conclusions

In summary, great care should be given to the alignment and assessment of non-coding sequence data. There is considerable evidence now that non-coding regions are highly structured, non-randomly evolving DNA; thus, alignment by current randomized algorithmic software is rarely adequate. An understanding of the proposed mechanisms of mutation acting on non-coding sequences is critical for the positioning of gaps and the better assessment of homology of indels and point substitutions. Probable secondary structure should be routinely identified and used as an important source of information to aid in aligning chaotic or labile regions of the data matrix. Prior to phylogenetic analysis, all matrices should be carefully reviewed for obscured mutational events, such as minute inversions or misaligned repeat units.

Important for understanding molecular evolution in non-coding DNA is the concept of the mutational trigger (Kelchner, 1996; Kelchner & Clark, 1997), a specific sequence pattern that creates the foundation for a mutational event. Such triggers often remain intact after generating a mutation, and their presence can easily occasion a repeated, paralleled, or reversed mutation event. Triggers may likely be responsible for much of the homoplasy of gap characters inferred in studies at any taxonomic level; those applying non-coding sequence data to molecular systematics should be aware of their occurrence and effect.

Information of the kind presented here can increase the predictive value of mutational events in non-coding DNA. For example, Kelchner and Wendel (1996) suggested that minute inversions associated with hairpin secondary structures described in non-coding cpDNA could occur in similar situations in other genomes. Dumolin-Lapègue et al. (1998) recently reported just such an event in the mitochondria of oak populations of southern France. Hence, recommendations proposed in this paper for the phylogenetic analysis of non-coding cpDNA sequences may likely apply to data from non-coding regions of nuclear, and particularly mitochondrial, genomes.

Choosing an appropriate non-coding region for a particular taxonomic level is essential for maximizing its utility as a phylogenetic tool, but there is no infallible method for determining what that "proper" degree of mutation is for a particular study. A region's utility may vary between plant groups that are assumed to occupy the same evolutionary level, and data from multiple non-coding regions, when applied to one taxonomic group, can vary remarkably in phylogenetic utility (see Small et al., 1998). In light of the mutational mechanisms outlined in this article, at least one concern seems justified: if the taxonomic level is too high, one would expect saturation of multiple hit sites and concealment of multiple hit indels in any non-coding region, decreasing its utility as a phylogenetic tool.

The perceived intricacies of molecular evolution and their bearing on phylogenetic analysis, both in non-coding and coding regions (for genes have well-known mechanistic biases as well—the codon position being just one example) can be discour-

aging. However, the phenomena outlined in this article have solutions in most cases, and attention to alignment and analysis should enhance the phylogenetic utility and accuracy of non-coding cpDNA data. It should be noted that in almost all systematic studies based on non-coding cpDNA sequences, the authors profess to have found sufficient phylogenetic information in their data to warrant its use in lower-level phylogenetic analyses.

Clearly there is a need to develop an understanding of molecular evolution in non-coding cpDNA regions similar to that which exists for chloroplast genic DNA. Continued research into non-coding sequence evolution may eventually produce a more balanced process for the alignment and phylogenetic analysis of non-coding sequence data. Future software may be able to measure and assess probabilities associated with particular mutational mechanisms and incorporate this information into the alignment process. This would be an immense aid to those systematists who wish to apply non-coding molecular tools to the field of plant systematics.

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ALLPAHUAYO: FLORISTICS, STRUCTURE, AND DYNAMICS OF A HIGH-DIVERSITY FOREST IN AMAZONIAN PERU[1]

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Abstract

This paper describes the results of a floristic inventory at the Allpahuayo Reserve, near Iquitos in Amazonian Peru. Two long-term one-hectare plots were established using a pre-determined sampling grid, with each individual tree and liana over 10 cm diameter collected at least once, except for palms. The plots were re-censused after 5 years to quantify forest dynamics. Floristic analysis shows that the Allpahuayo forest is among the most diverse site yet inventoried, with 281 to 311 species per hectare, and at least 466 species and 61 families in the 1277-stem two-hectare sample, confirming that upper Amazonia is a world center of tree biodiversity. The ecologically most dominant and speciose family in the plots is Fabaceae sensu lato, with 231 stems and 89 species; no other family represents more than 7% of the species or 10% of the stems. In contrast to the exceptional floristic diversity, both the structure and the dynamics of the Allpahuayo forest are similar to those recorded from other old-growth neotropical forests. Many tree and liana canopy species were previously unknown to both the Iquitos area and to Amazonian Peru, which demonstrates the significance of Amazon ecological studies to systematic botany.

Resumen

Este documento describe el estado actual de los resultados de un inventario florístico a largo plazo, en la Reserva Biológica de Allpahuayo, cerca de Iquitos, en la Amazonía Peruana. Allí se establecieron dos parcelas de una hect. cada una, usando un muestreo pre-determinado, cada árbol, palmera o liana, mayores o iguales que 10 cm de diám. fué marcado y colectado, excepto las palmeras que fueron colectadas solo una vez por especie. Las parcelas fueron re-censadas despues de 5 años para cuantificar el proceso de la dinámica del bosque. El análisis de los resultados nos muestra que el bosque de Allpahuayo, está entre los bosques mas diversos hasta ahora inventariados, con 281 a 311 especies por hect., y al menos 466 especies y 61 familias en 1277 individuos en dos hect. muestreadas; éstos datos apoyan la idea de que el oeste de la Amazonía es un centro mundial de diversidad de árboles. En las parcelas la familia Fabaceae s.l., es la mas dominante ecologicamente y en número de especies, contiene 231 tallos y 89 especies; ninguna otra familia representa más que el 7 por ciento de las especies o el 10 por ciento de los tallos. En contraste a la excepcional diversidad florística, tanto la estructura del bosque como la tasa de mortalidad en Allpahuayo, están dentro los rangos de otros sitios neotropicales. El valor de los estudios ecológicos para la botánica sistemática, se demuestra en tanto que, antes de que empezáramos a tomar datos ecológicos e instalar parcelas permanentes, muchas especies de árboles y lianas eran desconocidas tanto en el área de Iquitos, como en la Amazonía Peruana.

Key words: Amazonia, floristics, inventory, Neotropics, species richness, species turnover.

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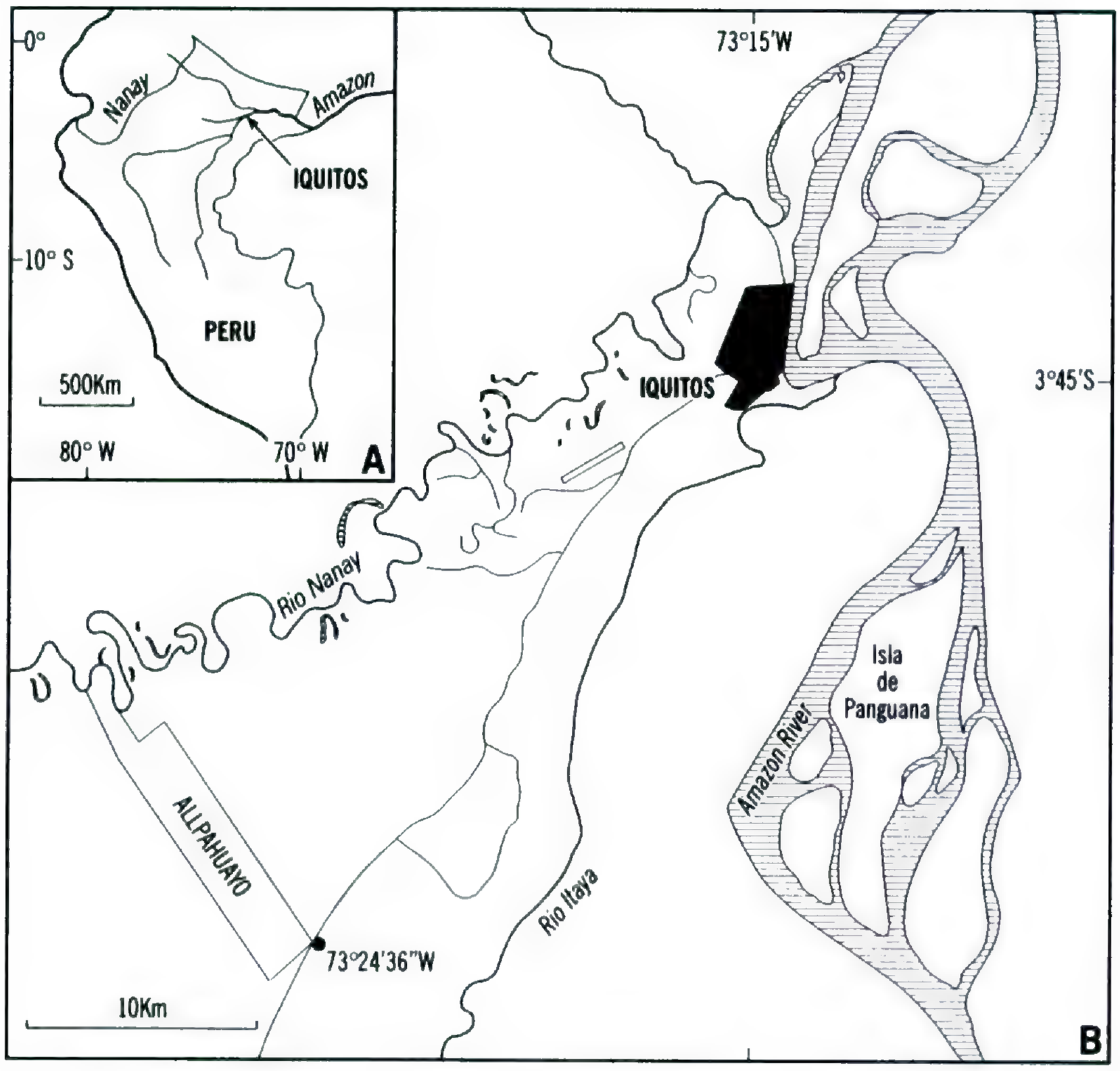

Figure 1. Map showing location of study region in relation to Iquitos and Peru. —A. Peru. —B. Iquitos region, showing location of Allpahuayo. Modified from Kalliola et al. (1998).

Biodiversity is at risk in much of the tropics. Yet biological research and conservation initiatives are hampered by inadequate baseline information. Thus, sufficient data on the numbers, kinds, and abundance of most major plant and animal taxa are not available. Moreover, knowledge of species associations and biogeographic distributions of taxa remain meager (McNeely et al., 1990; National Science Board, 1990; National Research Council, 1992; Phillips & Raven, 1996).

In the Neotropics, the extraordinary species richness, our limited taxonomic understanding, and the physical inaccessibility of most areas are formidable barriers to phytogeographic investigation. Traditional biological inventory efforts that rely mostly on ad hoc collection efforts and lengthy monograph studies cannot address the knowledge gap alone. Alternative methods are critical to improve our understanding of the factors determining species composition and the ecological dynamics of tropical forest ecosystems. In recent years there has been growing scientific interest in more ecological, plot-based work as a means of understanding tropical forests (e.g., Gentry, 1988a, b; Phillips & Gentry, 1994; Phillips et al., 1994; Dallmeier & Comiskey, 1998a, b). This approach can contribute greatly to floristic understanding by generating large numbers of new collections that are associated with site-specific ecological information. Botanical institutions play a vital role in initiating and supporting this work, and contribute essential expertise for ensuring accurate voucher determination. Equally, ecological inventories generate much biogeographical data of relevance to monographic and phylogenetic studies. Such eco-floristic research has recently been initiated in several tropical countries, including Madagascar (Lowry et al., 1997; Rakotomalaza & Messmer, 1999), Colombia (Rudas, 1996), and Peru (Gentry, 1988a, b). This paper reports the results of a similar research program at the Allpahuayo Reserve, near Iquitos in northern Peru.

Study Site

The Allpahuayo Reserve (3°57′S, 73°26′W) lies southwest of Iquitos in Amazonian Peru, between the blackwater Rio Nanay on the northwest and the Iquitos–Nauta road to the southeast (Fig. 1). This 2750-ha reserve is administered by the Peruvian Institute for Amazonian Research (IIAP). The climate is humid and hot (with the mean annual precipitation about 3000 mm and an average temper-

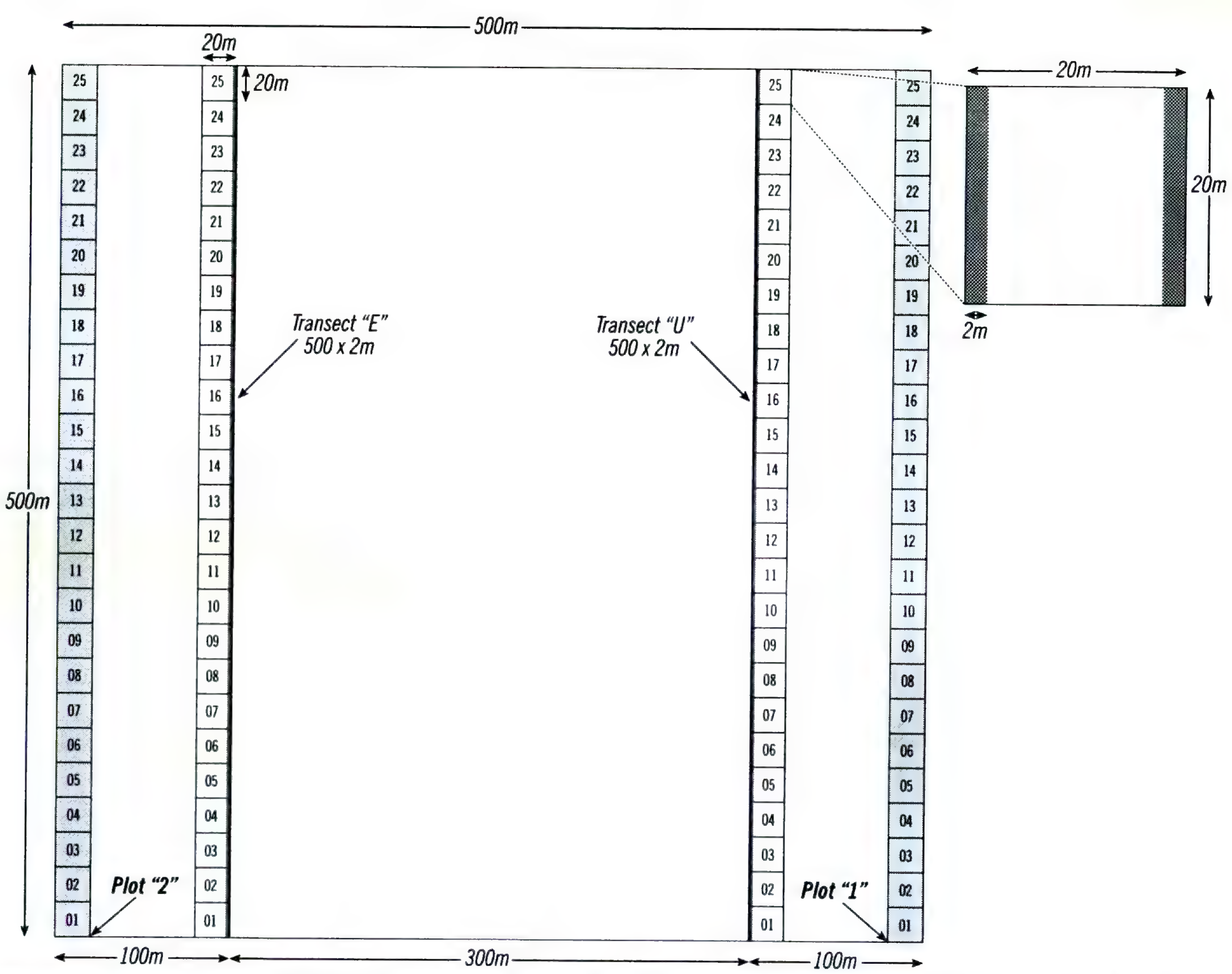

Figure 2. Diagram showing the arrangement of plots within a 500 × 500 m sampling grid at Allpahuayo. The permanent 500 × 20 m plots ("1" and "2") are located at the edges of the grid. Additional parallel temporary samples of 500 × 2 m were established 100 m in from the edges (Transects "E" and "U," shaded in the figure). For comparison we also show the relative positions of additional 500 × 20 m plots that incorporate transects "E" and "U" at other sites in Peru (Cusco Amazónico and Sucusari/ACEER) but not at Allpahuayo.

ature of 26°C; Marengo, 1998). Allpahuayo Reserve lies between 110 m and 180 m above sea level. Edaphic conditions are variable, representing a mosaic of patches ranging in texture from clayey to almost pure sand, and in drainage from waterlogged swamps to well-drained hill tops. Vegetation is mature, old-growth forest, although some palm trees (*Oenocarpus bataua* Mart.) have been cut to harvest their fruits. Allpahuayo and neighboring Mishana comprise one of three locations within 100 km of Iquitos where the Missouri Botanical Garden has been conducting a long-term floristic inventory effort with the institutional collaboration of IIAP and Explorama Tours S.A. (Vásquez, 1997). The other two sites, Reserva Ecológica Explorama Lodge (Yanamono) and Reserva Ecológica Sucusari (ACEER), have rather different soil conditions and floristic composition. The 3750 ha of Allpahuayo-Mishana is extremely species-rich, with more than 10% of the plant species reported from the whole of Peru (Brako & Zarucchi, 1993) recorded here (Vásquez, 1997).

Methods

Sampling took place within a 500 × 500 m grid in the Allpahuayo Reserve, sufficiently far from the Iquitos-Nauta road (> 2 km at its nearest point) to minimize human disturbance, yet close enough to the research station (3 km) to allow us to commute daily. Within the sampling grid, two long-term plots were established in a pre-determined location within the grid (Fig. 2) to eliminate subjective bias. Each plot is 20 × 500 m, with each plant ≥ 10 cm DBH (diameter at breast height, 1.3 m) marked with sequentially numbered aluminum tags. Additional narrower samples of 2 × 500 m with all plants ≥ 2.5 cm DBH collected but not tagged were made at pre-determined points in the grid: the results of these inventories will be reported in a future publication. The linear nature of all our plots means that they each traverse a mixture of edaphic formations. Soil conditions within the grid ranged from poorly to adequately drained clay soils ("Shapaja") to well-drained white sand soils at topographic high points ("Varillal").

In the long-term plots, every tagged plant's height was visually estimated and its diameter measured at 1.3 m with diameter tape. Herbarium collections (*Vásquez 14592–15829*) were made in 1990 and 1991 from every individual plant (except for palm tree species, which were only collected once each) using extendable aluminum collecting poles and, where necessary, spiked climbing irons for climbing trees. A full set of duplicates is deposited in two Peruvian herbaria (AMAZ, USM) and at MO, with partial collections existing at IIAP and the Universidad Agraria La Molina (MOL), as well as distributions to family specialists worldwide.

Fertile collections were made on occasion whenever flowers or fruits of plot species were seen, but in common with other tropical plot inventories most species (90%) are represented only by sterile material. At MO every collection number was first identified to family by the first author and Ron Liesner, with each family set then distributed to family specialists (Anacardiaceae, Apocynaceae, Bignoniaceae, Burseraceae, Chrysobalanaceae, Dilleniaceae, Fabaceae, Lauraceae, Lecythidaceae, Malpighiaceae, Melastomataceae, Meliaceae, Moraceae, Myrtaceae, Rubiaceae, Sabiaceae, Sapindaceae, Sapotaceae, Simaroubaceae, Violaceae). For most species at least three fertile Peruvian collections were noted for verification (MO), most deriving from the Iquitos Florula project (Vásquez, 1997).

Re-census of both plots was done in February 1996, with the re-measurement of all trees and lianas. Plants that had died in the intervening period of 5.25 years were noted as such, with evident cause of death. Each new recruit into the 10 cm DBH class was measured, tagged, and given a unique number. New recruits were collected and vouchers distributed to herbaria as for the original collections.

In order to maximize potential comparability with other forests, diversity values for each plot were expressed in terms of both species richness and Fisher's Alpha, using various subsets of our data. We estimated "species richness"—the sum of the number of tree species—as this measure is easily understood and widely reported from other forests. We also estimated Fisher's Alpha, where:

$$\text{species richness} = \text{Alpha} * \ln(1 + \text{stems}/\text{Alpha})$$

Fisher's Alpha values from small tropical forest tree samples provide good estimates of the overall diversity of each forest (Condit et al., 1998). Both species richness and Fisher's Alpha values were calculated on a per-area basis (i.e., for each one-hectare plot), and on an area-independent basis (i.e., for the first 500 stems encountered in each plot) in order to remove the complicating effect of varying stem density. Finally, each diversity index was also estimated separately for all woody stems, and for trees alone.

In order to compare stem densities of individual species and families, average per-hectare values were calculated. The most speciose families across

Table 1. Allpahuayo forest diversity in 1990, for stems ≥ 10 cm diameter in 1-ha plots. The values given for species richness and Fisher's Alpha are for the minimum and the most likely (underlined) estimates, respectively. The diversity values are calculated for (A) all woody stems (i.e., trees and lianas), and (B) for trees only. See text for further details.

	Stems	Species	Fisher's Alpha	Species per first 500 stems	Fisher's Alpha, first 500 stems
(A) All woody stems					
Plot 1	643	281/293	190/208	233/242	170/185
Plot 2	634	306/311	233/242	250/255	199/208
Plots 1 and 2 combined	1277	466/480	264/280	N/A	N/A
(B) Trees only					
Plot 1	616	264/275	175/191	228/237	162/176
Plot 2	608	290/295	218/226	243/248	186/195
Plots 1 and 2 combined	1224	433/444	239/250	N/A	N/A

our whole sample were tabulated on the basis of the total species recorded in both hectare plots. The degree of departure from randomness in species' distribution with respect to soil type was explored using a binomial test based on the relative frequency with which individuals were recorded in clay soil and sandy soil forests.

Standard measures of forest structure (density of stems and total basal area of stems ≥ 10 cm DBH) were computed for each hectare plot. Annual mortality and recruitment rates were estimated using standard procedures that use logarithmic models that assume a constant probability of mortality and recruitment through each inventory period (Phillips et al., 1994; Swaine & Lieberman, 1987), and computed separately both for woody stems and for woody basal area. Turnover rates for each period were represented by the mean of recruitment and mortality rates (Phillips, 1996).

Results and Discussion

(a) taxon identification

Of the 1277 plants in the plots in 1991, 100% were identified to family, 1249 (97.8%) to genus, and 1168 (91.5%) to species (Appendix 1). We made 1160 collections from the two plots, of which 1053 (90%) were sterile, 22 (2%) were in bud, 33 (3%) were in flower, and 52 (5%) were in fruit. The high proportion of sterile material complicated the identification process. Near-complete identification was possible only because of the large number of fertile collections from Allpahuayo and nearby localities available for comparison at MO. For most of the unidentified species, it was possible to allocate collections to morphospecies (i.e., to allocate collections to a morphological species concept, albeit unnamed), but for a few even this was not possible.

(b) species richness and diversity

Because of the difficulties with identification there is some uncertainty about the exact number of species in our samples. We estimated a "most likely value" for species richness ($\hat{S}$) by multiplying the number of unidentified plants per sample that could not be allocated to morphospecies (U) by the ratio of species:individuals for the plants in that sample that were identified to species or morphospecies (R), and adding this value to the number of morphospecies and species concepts actually distinguished (D).

Thus, $\hat{S} = (U * R) + D$.

This estimate probably errs on the conservative side because the most difficult plants to identify tend to be the rarest, and therefore it is likely that the ratio of species to individuals will be greater for non-identified plants than it is for identified plants.

Diversity values for each plot and for both plots combined are presented in Table 1, with results given separately for all stems and for trees alone. These reveal that the Allpahuayo plots are some of the richest 1-ha samples ever reported. The best estimate of Fisher's Alpha for trees in our plot 2 (226) is the greatest value ever recorded for trees ≥ 10 cm DBH in 1-ha plots, comparing with 221 at Yanamono in Peru (Condit et al., 1998), 211 at Cuyabeno in Ecuador (Condit et al., 1998), and 191 in Allpahuayo plot 1 (this study), all of which are upper Amazonian sites. In terms of published results of numbers of species per 500 stems, the All-

pahuayo plots are surpassed only by Yanamono (Phillips et al., 1994). There are also some published inventory results of, variously, lianas, hemiepiphytes, trees, and saplings from this part of Amazonia (e.g., Gentry, 1988a, b, 1992; Valencia et al., 1994; Clinebell et al., 1995; Duivenvoorden & Lipps, 1995). These confirm that the region's unflooded forests generally have globally exceptional levels of diversity for woody plants.

Within-community ("alpha-") diversity in neotropical forests is closely correlated with climatic conditions, with the richest forests found in the hot and aseasonal equatorial lowlands (Clinebell et al., 1995). High levels of local diversity may also be a consequence of close juxtaposition of different topographical and soil formations in western Amazonia (Tuomisto et al., 1995) with "mass-effect" processes causing species to spill over from adjacent edaphically defined communities. Each Allpahuayo plot traverses contrasting substrates, of sandy and clayey soils, which contributes to the high diversity values, as some species are apparently specialized on each particular soil type (cf. (c) below). However, the Allpahuayo plots were placed without regard to forest type, so these diversity values are presumably typical for most 1-ha patches within the Allpahuayo forest. Data are also available from one 0.1-ha sample of plants ≥ 2.5 cm DBH, purposively laid out at Allpahuayo by A. Gentry and the first author so as to only sample clay soil forest (Clinebell et al., 1995; *http://www.mobot.org/MOBOT/research/applied_research/gentry.html*). Here 275 species of trees, lianas, and hemiepiphytes were found in 401 individuals, giving a Fisher's Alpha value of 386, the highest species richness and Fisher's Alpha value of all Gentry's 227 0.1-ha samples worldwide (Phillips & Miller, in prep.).

(C) FLORISTIC COMPOSITION

The Allpahuayo forest is not only very species-rich, but also has a very low degree of dominance by any one species. The ten most common species together represent less than 20% of all stems (Table 2A). Most of the families that physically dominate the forest (Table 2B), such as Fabaceae, Myristicaceae, Euphorbiaceae, Sapotaceae, and Moraceae, do so by virtue of having many species almost all of which are present at a very low density. The palm family is atypical in that its status as the second-most stem-dense family is due mostly to the high density of one species (*Oenocarpus bataua* Mart.).

The most speciose families in our entire Allpahuayo sample (Table 2C) are the same families that dominate other Amazonian forests (Gentry, 1988a). Fabaceae are by far the most species-rich, and even when treated individually the three legume subfamilies each have about as many species as the other top-five largest families (Moraceae, Lauraceae, Annonaceae, Burseraceae).

The plots each traverse two contrasting soil types, richer clay soils (the "Shapaja" series) and poor white sand soils (the "Varillal" series), and the strong local edaphic contrasts might be expected to result in some floristic differentiation within the plots. Because of the high diversity, few species were sampled frequently enough in the two plots to allow us to test the extent to which they are habitat specialists or generalists. However, among the 56 species that were relatively common, with five or more individual plants in the two hectares, a binomial test reveals that a greater number are confined to one soil type or another than would be expected under a null model of random distribution (Table 3). This analysis reveals a large number of species that are apparent specialists on the poor white sand soils (for example, *Macrolobium microcalyx, Ocotea aciphylla, Tachigali ptycophysca,* Table 3A), and a smaller group mostly confined to the relatively rich clay soils (for example, *Leonia glycycarpa, Lindackeria paludosa, Senefeldera skutchiana,* Table 3B). Note that these results are not proof of specialization, since apparent specialists could arguably be constrained to one patch of forest by chance alone, especially if they have poor dispersal mechanisms. However, a parallel study at Allpahuayo (Vormisto et al., 2000) demonstrated that the local-scale distributional pattern of soil types is closely related to plant distributional patterns, not only for tree species but for other plant groups as well, and these spatial patterns are congruent among different groups. This research confirms that the local distributional patterns of many plants within the Allpahuayo plots are influenced by edaphic conditions. The high diversity of the Allpahuayo plots is therefore not only due to high intra-community diversity, but also derives from the contrasting edaphic conditions evident within each plot.

(D) STRUCTURE AND DYNAMICS

Although the Allpahuayo plots are exceptionally species-rich, they do not appear to be particularly remarkable in terms of their physical structure. This is indicated by the fact that both their stem density and tree basal area appear to be well within the typical ranges for most lowland Amazonian forests (Table 4). However, we could find no published

Table 2. Dominant woody plant taxa at Allpahuayo.

A. The 10 species with the highest density of stems ≥ 10 cm diameter per hectare, on average and by soil type. The two plots included 1.16 ha of clay soil forest, and 0.84 ha of sandy soil forest.

Family	Genus	Stem density	Stem density, clay soil forest	Stem density, sandy soil forest
Palmae	*Oenocarpus bataua* subsp. *bataua*	39.0	26.3	55.2
Myristicaceae	*Iryanthera ulei*	14.5	8.8	20.8
Myristicaceae	*Virola pavonis*	11.5	11.3	14.6
Euphorbiaceae	*Hevea guianensis*	10.0	1.3	18.8
Fabaceae	*Macrolobium microcalyx*	9.5	0	19.8
Annonaceae	*Diclinanona tessmannii*	7.5	0	15.6
Euphorbiaceae	*Micranda elata*	7.5	0	15.6
Euphorbiaceae	*Senefeldera skutchiana*	7.5	17.5	1.0
Apocynaceae	*Aspidosperma excelsum*	7.0	1.3	12.5
Palmae	*Astrocaryum macrocalyx*	7.0	16.3	1.0
Top 10 species		121.0	82.8	175.9
All species		638.5	637	640

B. The 10 families with the highest density of stems ≥ 10 cm diameter per hectare.

Family	Stem density
Fabaceae:	115.5
Caesalpiniaceae	53.0
Mimosaceae	23.5
Papilionaceae	36.5
Indet. Fabaceae	2.5
Palmae	61.5
Myristicaceae	59.0
Euphorbiaceae	56.5
Sapotaceae	33.0
Moraceae	30.5
Annonaceae	27.0
Lauraceae	26.5
Burseraceae	21.5
Lecythidaceae	16.0
Top 10 families	420.0
All families	638.5

C. The 10 families with the highest number of species ≥ 10 cm diameter sampled throughout our 2-ha sampled area. The values given are for the minimum and most likely (underlined) number of species per family.

Family	Total species inventoried
Fabaceae:	82/89
Caesalpiniaceae	27/29
Mimosaceae	25/28
Papilionaceae	28/29
Indet. Fabaceae	2/3
Moraceae	31/31
Lauraceae	29/29
Annonaceae	27/27
Burseraceae	23/24
Euphorbiaceae	23/23
Sapotaceae	22/23
Myristicaceae	21/21
Lecythidaceae	14/14
Myrtaceae	10/12
Top 10 families	262/273
All families	433/444

Table 3. Edaphic specialists in the Allpahuayo plots. For all species with five or more individuals, a binomial test was conducted to test the null hypothesis of random distribution with respect to soil type. The hypothesis was rejected for half the 56 species tested (* ($P < 0.05$); ** ($P < 0.01$); *** ($P < 0.001$)). See text for further details.

Family	Taxon	Total stems	Shapaja stems	Varillal stems	Probability of random distribution
A. Specialists on white sand ("Varillal" soils).					
Annonaceae	*Diclinanona tessmannii*	15	0	15	***
Apocynaceae	*Aspidosperma excelsum*	14	1	12	**
Bignoniaceae	*Jacaranda macrocarpa*	5	0	5	*
Euphorbiaceae	*Hevea guianensis*	20	1	18	***
Euphorbiaceae	*Micranda elata*	15	0	15	***
Fabaceae (Caes.)	*Macrolobium bifolium*	8	0	8	**
Fabaceae (Caes.)	*Macrolobium microcalyx*	19	0	19	***
Fabaceae (Caes.)	*Sclerolobium bracteosum*	5	0	5	*
Fabaceae (Caes.)	*Tachigali ptychophysca*	10	0	10	***
Fabaceae (Caes.)	*Tachigali tesmannii* cf.	13	0	13	***
Fabaceae (Pap.)	*Swartzia racemosa* var. *klugii*	11	1	10	**
Icacinaceae	*Emmotum floribundum*	5	0	5	*
Lauraceae	*Ocotea aciphylla*	8	0	8	**
Malpighiaceae	*Byrsonima stipulina*	8	0	8	**
Myristicaceae	*Iryanthera ulei*	29	7	20	*
Nyctaginaceae	*Neea floribunda*	8	1	7	*
Palmae	*Oenocarpus bataua* subsp. *bataua*	78	21	53	***
Rosaceae	*Prunus detrita* vel sp. aff.	5	0	5	*
Rubiaceae	*Ferdinandusa chlorantha*	5	0	5	*
Sapotaceae	*Chrysophyllum bombycinum*	9	0	9	**
B. Specialists on clay ("Shapaja" soils).					
Euphorbiaceae	*Conceveiba rhytidocarpa*	7	6	1	*
Euphorbiaceae	*Nealchornea yapurensis*	9	8	1	**
Euphorbiaceae	*Senefeldera skutchiana*	15	14	1	***
Flacourtiaceae	*Lindackeria paludosa*	6	5	0	*
Monimiaceae	*Siparuna decipiens*	5	5	0	*
Palmae	*Astrocaryum macrocalyx*	14	13	1	***
Palmae	*Iriartea deltoidea*	13	12	1	***
Violaceae	*Leonia glycycarpa* var. *glycycarpa*	8	6	0	*

Table 4. Stem density and basal area of some lowland Amazonian forests, for stems ≥10 cm diameter per ha. Data are from this study (Allpahuayo, 1990 census) or other sources (Mishana, Tambopata, Yanamono: Gentry, 1988b; Añangu, Jatun Sacha, Belém, San Carlos de Rio Negro: Phillips et al., 1994; Cusco Amazónico: Phillips et al., unpublished data). Stem density values are per ha. Basal area values are m^2 per ha.

Site	Tree stem density	Liana and strangler stem density	Tree basal area	Liana and strangler basal area
Belém, Brazil	572		27.7	
Jatun Sacha, Ecuador	724		30.5	
Añangu, Ecuador	734		23.1	
Allpahuayo Plot 1, Peru	616	27	26.77	0.42
Allpahuayo Plot 2, Peru	608	26	27.33	0.27
Cusco Amazonico Plot 1, Peru	489	45	25.9	
Mishana, Peru	842	16	28.7	
Tambopata Plot 1, Peru	585	17	26.9	
Yanamono, Peru	580	26	32.7	
San Carlos de Rio Negro, Venezuela	744		23.0	

Table 5. Annual rates of natural stem and basal area mortality, recruitment, and increment, for stems ≥ 10 cm diameter, between 1990 and 1996. All results are based on 0.84 ha (plot 1) and 0.96 ha (plot 2).

A. Stem dynamics.

	Annual mortality	Annual recruitment	Annual turnover (mean of loss and gain)
Plot 1: Tree stems	1.89%	1.57%	1.73%
Plot 2: Tree stems	1.84%	2.04%	1.94%
Plot 1: Liana and strangler stems	4.41%	1.89%	3.15%
Plot 2: Liana and strangler stems	4.87%	8.02%	6.44%

B. Basal area dynamics. Note that the annual gain in basal area is the sum of new recruitment into the 10 cm diameter size-class, plus the growth of pre-existing stems ≥ 10 cm diameter, and annual turnover is the mean of mortality and gain rates.

	Annual mortality	Annual recruitment	Annual gain	Annual turnover
Plot 1: Tree basal area	1.76%	0.48%	2.08%	1.92%
Plot 2: Tree basal area	2.21%	0.57%	2.32%	2.26%
Plot 1: Liana and strangler basal area	5.69%	1.03%	5.72%	5.71%
Plot 2: Liana and strangler basal area	8.29%	7.24%	8.57%	8.43%

data for liana basal area (lianas are rarely systematically censused in ecological plots, and liana basal area is even more rarely reported), so it is not possible to compare Allpahuayo with other forests in this respect. Elsewhere (Phillips et al., 1998) we reported that tree basal area values have been increasing in the majority of Amazonian plots censused since the mid-1970s, which we interpreted as being a possible effect of long-term fertilization by rising atmospheric concentrations of carbon dioxide. In this context it is interesting to note that by 1996, both plots at Allpahuayo had experienced small net increases in basal area (by 0.1% and 1.9%) over the 1990 values shown in Table 4, in spite of some illegal felling of palm trees within a few of the sub-plots. Clearly a longer census interval will be needed to confirm whether the small change in forest structure is part of a long-term trend at Allpahuayo, or simply part of a pattern of random fluctuation around a long-term stable state.

To estimate annual natural mortality and growth rates we excluded the sub-plots where palm trees were cut (Table 5). Stem turnover and basal area turnover functions measure slightly different attributes of the stand dynamics—stem turnover is concerned with population dynamics (i.e., the mean of population mortality and recruitment rates), while basal area turnover is concerned with basal area dynamics (i.e., the mean of basal area mortality and recruitment plus growth of existing trees). Over the long-term an old-growth forest would be expected to have similar values of each, and at Allpahuayo the turnover rates of tree stems and basal area both averaged close to 2% per year in the first half of the 1990s. This rate is not unusual by the standards of western Amazonian forests, but it is higher than the average turnover rate for other tropical moist forests (≈ 1.5% per year based on studies at 40 different sites; Phillips, 1996). Based on our small sample of lianas and stranglers, large climbing plants appear to turn over notably faster than trees, about 5% a year, which indicates that these organisms may have shorter canopy residence times than most trees. Whether this pattern is repeated elsewhere remains to be seen, but if faster turnover of lianas and stranglers is a general property of Amazon forests it would have implications for plot studies, most of which still ignore climbers. We may be overlooking a component of the forest that is more significant ecologically than has been appreciated.

Conclusions

Our floristic and ecological results at Allpahuayo well demonstrate the ecological and systematic benefits that can result when we concentrate our joint efforts on inventories in fixed plots. For the ecologist the benefits are clear—without the collaboration of botanists in the field and the herbarium it is impossible to characterize patterns of diversity and floristic composition in most tropical forests, let alone explore the factors that determine these. As forests become more vulnerable to widespread environmental stresses such as fragmentation and climate change (Laurance et al., 1997; Phillips,

1997), plots are also needed to monitor these impacts on biodiversity. Systematists at botanical gardens therefore offer essential expertise for understanding the biological effects of global change.

For the systematist or floristic monographer, establishing permanent sample plots can help in understanding the local flora. The precise rigor of ecological sampling forces researchers to look equally at every plant that meets pre-determined criteria. In contrast, peripatetic botanizing may catalog the weedy, common, obvious, and accessible species, while missing rarer or larger plants, especially canopy trees, lianas, and epiphytes. Rigorous plot inventories can therefore reduce the spatial, taxonomic, life-form, and even seasonal biases prevalent in herbaria (e.g., Nelson et al., 1990), especially when integrated into a larger intensive collecting effort. Thus, a comparison of taxa in Vásquez (1997) with those listed in Brako and Zarucchi (1993) shows that the intensive collecting effort in permanent plots and surrounding forest at several Iquitos sites has yielded nearly 250 taxa new to Peru. At Allpahuayo, new tree taxa have been recognized in Annonaceae (2 species; Chatrou, 1998) and in Lauraceae (3 species including a new genus; van der Werff, 1993, 1997), while the repetition of plot visits has also allowed us to collect fertile material confirming new herb and shrub taxa (e.g., Kallunki, 1994; Vásquez, 1997).

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Appendix 1. Woody plant taxa ≥10 cm DBH recorded from two 1-ha plots at Allpahuayo. All taxa are trees unless indicated as *** (liana). When a voucher is fertile, we indicate its status by the following code: bd (buds), fl (flowers), ft (fruits). See text for further discussion.

Family	Taxon and authority	No. plants in 2-ha	Vásquez collections (only the first six are listed per taxon)				
Anacardiaceae	*Tapirira peckoltiana* Engl.	2	14,608	14,614 bd			
	Tapirira retusa Ducke	1	15,185				
	Tapirira sp.	1	15,794				
Annonaceae	*Anaxagorea brevipes* Benth.	1	14,815 fl				
	Anaxagorea phaeocarpa Mart.	2	14,655 bd	14,757			
	Annona duckei Diels	1	15,614				
	Annona montana Macfad.	2	14,788	15,332			
	Annona poepipigii (Mart.) Maas & Westra	1	14,695				
	Bocageopsis mattogrossensis (R. E. Fr.) R. E. Fr.	1	15,324				
	Diclinanona tessmannii Diels	15	14,819	14,840	14,899	14,907	14,919	14,928
	Duguetia cauliflora R. E. Fr.	1	15,035 ft				
	Duguetia sp.	1	15,770				
	Fusaea longifolia (Aubl.) Saf.	2	14,764 bd	15,788 fl			
	Fusaea sp.	1	15,730				
	Guatteria elata R. E. Fr.	1	15,566				
	Guatteria megalophylla Diels	1	15,039				
	Guatteria olivacea R. E. Fr.	2	14,773	14,797 ft			
	Guatteria rugosa R. E. Fr.	1	14,943				
	Guatteria schomburgkiana Mart.	2	14,770	14,812 fl			
	Guatteria sessilis Fries	2	14,844	15,204			
	Oxandra xylopioides Diels	2	15,489 fl	15,802			
	Rollinia cuspidata Mart.	1	14,877				
	Rollinia peruviana Diels	4	14,776 fl	14,869	15,203 fl	15,671	
	Rollinia pittieri Saff.	1	14,643 fl				
	Unonopsis floribunda Diels	2	14,793	15,042			
	Unonopsis spectabilis Diels	1	15,569				
	Xylopia barbata Mart.	2	15,259	15,268			
	Xylopia benthamii R. E. Fr.	1	15,622 ft				
	Xylopia excellens R. E. Fr.	1	14,897				
	Xylopia nitida Dunal	1	15,536 bd				
	Xylopia sericea A. St.-Hil.	1	15,799 ft				

Appendix 1. Continued.

Family	Taxon and authority	No. plants in 2-ha	Vásquez collections (only the first six are listed per taxon)				
Apocynaceae	*Ambelania occidentalis* Zarucchi	2	15,107 ft				
	Aspidosperma desmanthum Benth. ex Müll. Arg.	2	15,251	15,425			
	Aspidosperma excelsum Benth.	14	14,823	14,825	14,831	14,839	14,852
	Aspidosperma spruceanum Benth. ex Müll. Arg.	1	14,818				
	Aspidosperma schultesii Woodson	1	15,341				
	Couma macrocarpa Barb. Rodr.	1	15,017				
	Himatanthus succuba (Spruce ex Müll. Arg.) Woodson	1	15,676				
	Malouettia killipii Woodson	2	14,601	14,622			
	Malouettia tamaquirina var. *tamaquirina* (Aubl.) A. DC.***	1	14,882				
	Odontadenia puncticulosa (Richard) Pulle***	2	15,407	15,504			
	Parahancornia peruviana Monachin	3	14,985	15,478	15,762		
Aquifoliaceae	*Ilex andarensis* vel sp. aff. Loes.	4	14,941	15,256 fl	15,427	15,435	
Araliaceae	*Dendropanax umbellatus* (Ruiz & Pav.) Decne.	2	14,908	15,440			
	Schefflera morototoni (Aubl.) Maguire, Steyerm. & Frodin	1	14,858	15,009			
Bignoniaceae	*Jacaranda macrocarpa* Bureau & K. Schum.	5	14,862	14,878	14,998	15,019	15,434
	Jacaranda obtusifolia subsp. *obtusifolia* Humb. & Bonpl.	1	15,015				
	Memora cladotricha Sandwith	1	15,485				
Bombacaceae	*Eriotheca macrophylla* subsp. *sclerophylla* (Ducke) Robyns	2	15,514				
	Matisia bracteolosa Ducke	2	15,620	15,662			
	Matisia hirta Cuatrec.	1	15,060				
	Matisia malacocalyx (A. Robyns & S. Nilsson) W. S. Alverson	2	15,199	15,499			
	Matisia soegengii Cuatrec.	1	15,733				
	Pachira insignis (Sw.) Sw. ex Savigny	5	14,881	15,113	15,182	15,598	15,702
	Phragmotheca mammosa subsp. *amazonica* W. S. Alverson	1	15,513				

Appendix 1. Continued.

Family	Taxon and authority	No. plants in 2-ha	Vásquez collections (only the first six are listed per taxon)					
Boraginaceae	*Cordia mexiana* I. M. Johnst.	1	15,135					
	Cordia nodosa Lam.	2	14,624	15,660				
Burseraceae	*Crepidospermum goudotianum* (Tul.) Triana & Planch.	2	15,607	15,653				
	Crepidospermum prancei Daly	1	15,771					
	Dacryodes peruviana (Loes.) J. F. Macbr.	1	15,766					
	Protium aracouchini (Aubl.) Marchand	1	15,500					
	Protium crassipetalum Cuatrec.	1	15,822					
	Protium divaricatum Engl.	1	15,785					
	Protium ferrugineum (Engl.) Engl.	1	15,045					
	Protium fimbriatum Swart	3	15,176	15,172	15,684			
	Protium gallosum Daly	1	14,744					
	Protium grandifolium Engl.	6	15,179	15,308	15,492	15,574	15,599	15,778
	Protium hebetatum Daly	5	14,634	14,895 fl	14,898	15,563	15,567	
	Protium macrophylla (HBK) Engl.	1	15,642					
	Protium nitidifolium Daly	2	14,787	15,760				
	Protium nodulosum Swart	1	15,650					
	Protium opacum Swart subsp. *opacum*	2	14,599	14,612	15,521	15,797		
	Protium opacum vel sp. aff. Swart	2	15,521	15,797				
	Protium subserratum (Engl.) A. DC. & C. DC.	1	14,975					
	Protium tenuifolium vel sp. aff. (Engl.) Engl.	1	15,720					
	Protium trifoliatum Engl.	1	14,724					
	Protium sp. aff. *robustum*	1	15,764					
	Protium sp.	4	15,074	15,094	15,125	15,171		
	Tetragastris panamensis (Engl.) Kuntze	1	15,136					
	Trattinickia peruviana Diels	2	14,775	15,442				
Capparidaceae	*Capparis schunkei* J. F. Macbr.	1	15,806 fl					
Caryocaraceae	*Anthodiscus klugii* Standl. ex Prance	1	15,525					
	Anthodiscus pilosus Ducke	1	15,395					
	Caryocar glabrum (Aubl.) Pers.	1	15,801					
	Caryocar harlingii Prance & Encarn.	2	14,802	15,201				
Celastraceae	*Maytenus amazonica* Mart.	1	14,843					

Appendix 1. Continued.

Family	Taxon and authority	No. plants in 2-ha	Vásquez collections (only the first six are listed per taxon)			
Chrysobalanaceae	*Couepia dolichopoda* Prance	1	15,284			
	Couepia guianensis Aubl. subsp. *guianensis*	1	14,659			
	Couepia racemosa Benth. ex Hook. f.	1	14,783			
	Licania arachnoidea Fanshawe	1	14,777			
	Licania blackii Prance	1	15,080			
	Licania canescens Benoist	1	15,079			
	Licania heteromorpha var. *heteromorpha* Benth.	2	14,979	14,984		
	Parinari klugii Prance	1	15,206			
	Parinari parilis J. F. Macbr.	1	15,606			
Clusiaceae	*Caraipa punctulata* Ducke	2	14,731	14,808 fl		
	Chrysochlamys weberbaueri Engl.	1	15,488 ft			
	Clusia laxiflora (Poepp.) D. Dietr.	1	15,625			
	Moronobea coccinea Aubl.	1	15,198			
	Symphonia globulifera L. f.	1	15,800			
	Tovomita speciosa Ducke	3	14,732	15,148 ft	15,640	
	Tovomita stigmatosa Planch. & Triana	4	14,656	15,659	15,693	15,765
Combretaceae	*Buchenavia amazonica* Alwan & Stace	2	15,243	15,739		
Convolvulaceae	*Dicranostyles scandens* Benth.***	1	15,093			
Dichapetalaceae	*Tapura amazonica* Poepp.	1	14,673			
	Tapura coriacea J. F. Macbr.	1	15,084			
Dilleniaceae	*Doliocarpus amazonicus* Sleumer subsp. *amazonicus****	2	15,063	15,103		
	Doliocarpus novogranatensis Kubitzki***	1	14,763			
	Pinzona coriacea Mart. & Zuccagni***	1	14,680			
Ebenaceae	*Diospyros subrotata* Hiern	1	15,561 fl			
	Lissocarpa stenocarpa Steyerm.	2	15,447	15,470		

Appendix 1. Continued.

Family	Taxon and authority	No. plants in 2-ha	Vásquez collections (only the first six are listed per taxon)				
Elaeocarpaceae	*Sloanea durissima* Spruce ex Benth.	3	14,609	15,078	15,152		
	Sloanea floribunda Spruce ex Benth.	3	14,920	15,083	15,306		
	Sloanea guianensis (Aubl.) Benth.	2	14,888	15,631			
	Sloanea latifolia (Rich.) K. Schum.	1	15,753				
	Sloanea laxiflora Spruce ex Benth.	1	14,894 fl				
	Sloanea robusta Uittien	5	14,632	14,721	15,143	15,147	15,748
	Sloanea sinemariensis Aubl.	1	15,307				
	Sloanea tuerckheimii Donn. Sm.	1	15,803				
	Sloanea sp.	1	15,304				
Euphorbiaceae	genus	1	14,880				
	Alchornea schomburgkii Klotzsch	1	15,593				
	Alchornea triplinervia (Spreng.) Muell. Arg.	3	14,729	14,771 ft	15,011		
	Alchorneopsis floribunda (Benth.) Muell. Arg.	1	15,302				
	Conceveiba martiana Baill.	1	15,741				
	Conceveiba rhytidocarpa Muell. Arg.	7	14,611	14,630	14,738	15,560	15,600	15,617
	Conceveiba sp. nov.	3	15,633 fl	15,690	15,757		
	Dodecostigma amazonicum Ducke	1	14,700				
	Glycidendron amazonicum Ducke	1	15,048				
	Hevea guianensis Aubl.	20	14,704	14,817	14,821	14,822	14,824	14,850
	Hevea pauciflora (Spruce ex Benth.) Muell. Arg.	6	14,667 ft	14,887 ft	15,004	15,298	15,323	15,327
	Hyeronima alchorneoides Allemao	3	14,613 bd	14,616 bd	14,681		
	Hyeronima oblonga Cuatrec.	7	14,642	14,683	14,684	14,734	14,747	15,196 fl
	Mabea maynensis Muell. Arg.	3	15,071	15,480	15,334		
	Micranda elata Muell Arg.	15	15,250 ft	15,271	15,273	15,333	15,340	15,342
	Micranda spruceana (Baill.) R. E. Schult.	11	15,005	15,026	15,047	15,065	15,275 bd	15,265 fl
	Nealchornea yapurensis Huber	9	14,794	15,575	15,592	15,618	15,673 ft	15,678
	Pera benensis Rusby	1	15,274				
	Pera bicolor (Klotzsch) Muell. Arg.	1	15,424				
	Pera leandri vel sp. aff. Baill.	1	15,647				
	Plukenetia polyadenia Muell. Arg.***	1	15,651				
	Richeria grandis Vahl	3	14,678	14,913	15,501		
	Senefeldera skutchiana Croizat	15	14,596	14,646	14,657	14,660 ft	14,662	14,663

Appendix 1. Continued.

Family	Taxon and authority	No. plants in 2-ha	Vásquez collections (only the first six are listed per taxon)					
Fabaceae	genus	1	15,825					
	genus	1	15,681					
	genus	1	15,515					
	genus	1	15,538					
	genus	1	15,624					
Fabaceae: Caesalpinaceae	*Bauhinia guianensis* Aubl. subsp. *guianensis***	4	14,636	14,710	15,594	14,728		
	Bauhinia guianensis subsp. *kunthiana* (Vogel)***	3	14,785	14,918	14,933	15,002		
	Cynometra bauhiniifolia Benth.	4	15,551	15,573 ft	15,700 ft	15,745		
	Cynometra spruceana var. *spruceana* (Hayne) J. F. Macbr.	5	14,708	15,123	15,156	15,635	15,682	
	Dialium guianense (Aubl.) Sandwith	4	14,730	15,103A ft	15,486	15,818		
	Dimorphandra macrostachya Benth.	1	14,845					
	Hymenaea oblongifolia Huber	3	14,718	15,162	15,602			
	Macrolobium acaciifolium (Benth.) Benth.	1	14,931					
	Macrolobium angustifolium (Benth.) R. S. Cowan	2	14,689	14,837				
	Macrolobium arenarium Ducke	1	15,197					
	Macrolobium bifolium (Aubl.) Pers.	8	14,860	14,963	14,974	15,252	15,352	15,388
	Macrolobium limbatum Spruce ex Benth. var. *limbatum*	3	15,608	15,680	15,704	15,299		
	Macroloboium limbatum var. *propinquum* R. S. Cowan	1	15,299					
	Macrolobium microcalyx Ducke	19	15,244 ft	15,254	15,345	15,350	15,353	15,357
	Macrolobium sp.	1	15,301					
	Macrolobium sp.	1	15,339					
	Sclerolobium bracteosum Harms	5	15,305	15,310	15,363	15,391	15,461	
	Sclerolobium micropetalum Ducke	1	15,773					
	Sclerolobium vasquezii Pipoly	1	15,634					
	Senna sp.	1	15,564					
	Tachigali cavipes (Spruce ex Benth.) J. F. Macbr.	1	15,338 fl					

Appendix 1. Continued.

Family	Taxon and authority	No. plants in 2-ha		Vásquez collections (only the first six are listed per taxon)				
Fabaceae: Caesalpinaceae	*Tachigali chrysophylla* (Poepp.) Zarucchi & Herend.	1	14,799					
	Tachigali paniculata Aubl.	1	15,717					
	Tachigali poeppigiana Tul.	1	14,804					
	Tachigali ptychophysca Spruce ex Benth.	10	15,246	15,354	15,356	15,362	15,373	15,377
	Tachigali rusbyi Harms	1	15,611					
	Tachigali tessmannii cf. Harms	13	14,917	14,921	14,942	14,944	15,346	15,349
	Tachigali sp.	1	14,996					
	Tachigali sp.	1	15,030					
	Tachigali sp.	1	14,953					
	Tachigali sp.	1	14,957					
	Tachigali sp.	1	14,965					
	Tachigali sp.	1	14,961					
	Tachigali sp.	1	14,966					
	Tachigali sp.	1	14,856					
	Tachigali sp.	1	14,859					
	Tachigali sp.	1	14,871					
	Tachigali sp.	1	14,872					
Fabaceae: Mimosaceae	*Albizia* sp.	1	15,664					
	Enterolobium barnebeyanum Mesquita & M. F. Silva	1	15,562					
	Inga acrocephala Steud.	1	15,558					
	Inga bourgonii (Aubl.) DC.	1	15,619					
	Inga capitata Desv.	1	15,109					
	Inga coruscans Humb. & Bonpl. ex Willd.	1	14,604					
	Inga densiflora Benth.	1	14,772					
	Inga laurina (Sw.) Willd.	2	15,311	15,312				
	Inga multijuga Benth.	1	15,719					
	Inga oerstediana Benth.	1	15,559					
	Inga pezizifera Benth.	2	14,810	15,493				

Appendix 1. Continued.

Family	Taxon and authority	No. plants in 2-ha	Vásquez collections (only the first six are listed per taxon)				
Fabaceae: Mimosaceae	*Inga poeppigiana* Benth.	1	15,811				
	Inga semialata (Vell.) Mart.	1	15,537				
	Inga spectabilis var. *schimpfii* (Harms) Little	1	15,545				
	Inga velutina Willd.	1	15,698				
	Inga sp.	1	15,755				
	Inga sp.	1	15,523				
	Inga sp.	1	not collected				
	Inga sp.	1	14,598				
	Marmaroxylon basijugum (Ducke) L. Rico	4	15,024	15,776	15,792	15,804	
	Parkia igneiflora Ducke	3	14,991	15,124	15,138		
	Parkia multijuga Benth.	2	14,970	15,604			
	Parkia nitida Miq.	5	14,605	14,610	14,832	15,247	15,476
	Parkia panurensis Benth. ex H. C. Hopkins	1	voucher lost				
	Parkia velutina Benoist	2	15,260	15,335			
	Pithecellobium sp.	1	14,595				
	Pithecellobium sp.	1	14,650				
	Pithecellobium sp.	1	14,651				
	Pithecellobium sp.	1	14,692				
	Pithecellobium sp.	1	15,131				
	Pithecellobium sp.	1	15,502				
	Pithecellobium sp.	1	14,652				
	Stryphnodendron polystachyum (Miq.) Kleinhoonte	2	14,972	15,568			
	Zygia latifolia (L.) Fawcett	1	14,626				
Fabaceae: Papilionaceae	*Andira macrothyrsa* Ducke	2	15,548	15,627			
	Batesia floribunda Spruce ex Benth.	2	15,044	15,705			
	Dalbergia monetaria var. *monetaria* L. f.***	5	14,676A	14,756A	15,401	15,402	15,813
	Dalbergia riedelii (Benth.) Sandwith***	1	15,761				
	Dalbergia sp.***	1	15,064				
	Diplotropis purpurea var. *leptophylla* (Rich.) Amshoff	3	14,654	15,399	15,404		
	Dipteryx micrantha Harms	2	14,762	15,166			
	Hymenolobium nitidum Benth.	3	14,967	14,989	15,322		
	Lonchocarpus spiciflorus C. Mart. ex Benth.	1	15,375				
	Machaerium sp. cf. *multifoliatum****	1	15,498				
	Machaerium cuspidatum Kuhlm. & Hoehne***	1	15,657				

Appendix 1. Continued.

Family	Taxon and authority	No. plants in 2-ha	Vásquez collections (only the first six are listed per taxon)				
Fabaceae: Papilionaceae	*Machaerium floribundum* var. *parviflorum* Benth.***	1	14,627				
	Machaerium kegelli Meisn.***	1	15,810				
	Machaerium multifoliatum Ducke***	3	14,600	15,294	14,988		
	Machaerium sp.***	1	15,073				
	Machaerium sp.***	1	15,025				
	Machaerium sp.***	1	15,095				
	Ormosia arborea (Vell.) Harms	1	14,937				
	Pterocarpus amazonum (C. Mart. ex Benth.) Amshoff	1	14,929				
	Pterocarpus rohrii M. Vahl.	1	15,013				
	Swartzia benthamiana Miq. var. *benthamiana*	8	14,768	15,032	15,261 fl	15,292	15,628 bd	15,812 fl
	Swartzia brachyrachis var. *peruviana* R. S. Cowan	1	15,722				
	Swartzia cardiosperma Spruce ex Benth.	4	14,784	15,151	15,257 fl	15,347	
	Swartzia laevicarpa Amshoff	1	15,116				
	Swartzia polyphylla DC.	2	15,281	15,713			
	Swartzia racemosa var. *klugii* R. S. Cowan	11	14,765 bd	14,834	14,841	14,993	14,994	15,258 fl
	Swartzia tessmannii Harms	4	14,670	14,707	14,774	15,285	
	Swartzia sp.	1	15,061				
	Swartzia sp.	1	14,875				
	Taralea oppositifolia Aubl.	3	14,874	14,940	14,977		
	Vantanea parviflora Lam.	3	15,772	15,789	15,824		
Flacourtiaceae	*Casearia acuminata* DC.	1	15,177				
	Casearia combaymensis Tul.	1	14,751				
	Casearia fasciculata (Ruiz & Pav.) Sleumer	4	14,769	15,540 ft	15,552	15,686	
	Casearia javitensis Kunth	1	14,699 ft				
	Casearia sylvestris Sw. var. *sylvestris*	1	15,508 ft				
	Casearia ulmifolia M. Vahl ex Vent.	1	15,645				
	Lindackeria paludosa (Benth.) Gilg.	6	14,691	14,693 ft	15,104	15,694	15,715 ft	15,738
	Mayna grandifolia (H. Karst.) Warb.	1	15,056				
	Tetracylacium macrophyllum Poepp.	1	15,509				
	Tetrastylidium peruvianum Sleumer	7	14,798	14,827	15,150 ft	15,528	15,595 bd	15,605
Hippocrateaceae	*Salacia* sp.***	1	15,629				
	Tontelea ovalifolia subsp. *richardii* (Miers) A. C. Sm.***	1	14,676				

Appendix 1. Continued.

Family	Taxon and authority	No. plants in 2-ha	Vásquez collections (only the first six are listed per taxon)					
Humiriaceae	*Sacoglottis ceratocarpa* Ducke	1	14,615					
	Sacoglottis sp.	1	15,511					
Icacinaceae	*Dendrobangia boliviana* Rusby	1	15,145					
	Discophora guianensis Miers	1	15,422					
	Emmotum floribundum Howard	5	14,950	14,951	15,336	15,348	15,437	
Lacistemataceae	*Lacistema aggregatum* (Bergius) Rusby	4	14,594 ft	14,597	15,531	15,736 ft		
Lauraceae	genus	1	15,547					
	genus	1	15,663					
	genus	1	15,674					
	genus	1	15,643					
	Anaueria brasiliensis Kosterm.	2	14,742	14,786				
	Aniba perutilis Hemsl.	4	14,959	15,253	15,413	15,454		
	Aniba taubertiana Mez	1	15,139 ft					
	Endlicheria citriodora van der Werff	2	14,922	14,949				
	Licaria brasiliensis (Nees) Kosterm.	1	15,496					
	Licaria canella (Meisn.) Kosterm.	2	14,838	15,768				
	Licaria sp. cf. *dolichantha* Kurz	1	15,280					
	Licaria sp. cf. *rodriguesii* Kurz	1	15,570					
	Mezilaurus opaca Kubitzki & van der Werff	2	15,746	15,759 ft				
	Nectandra acuminata (Nees) J. F. Macbr.	1	15,621					
	Nectandra globosa (Aubl.) Mez	2	15,099	15,313				
	Nectandra pearcei Mez	1	15,731					
	Ocotea aciphylla (Nees) Mez	8	15,245	15,290	15,416	15,417	15,432	15,436
	Ocotea argyrophylla Ducke	4	14,932	14,925	14,936	14,939		
	Ocotea bofo Kunth	4	15,059	15,117	15,729	15,815		
	Ocotea bracteosa (Meisn.) Mez	1	15,455					
	Ocotea leucoxylon (Sw.) Laness.	1	15,418					
	Ocotea longifolia Kunth	1	15,632 ft					
	Ocotea myriantha (Meisn.) Mez	2	14,833	15,325				
	Ocotea puberula (Rich.) Nees	2	15,370	15,361				
	Ocotea whitei Woodson	1	15,140					
	Ocotea sp.	1	15,821					

Appendix 1. Continued.

Family	Taxon and authority	No. plants in 2-ha	Vásquez collections (only the first six are listed per taxon)					
Lauraceae	*Ocotea* sp.	1	15,751					
	Persea sp. cf. *boliviensis*	1	15,467					
	Pleurothyrium vasquezii van der Werff	1	15,649					
Lecythidaceae	*Cariniana* sp. cf. *multiflora* Ducke	1	14,753					
	Eschweilera chartaceifolia S. A. Mori	3	14,750	14,906	15,112			
	Eschweilera coriacea (DC.) S. A. Mori	7	14,641	15,057	15,062	15,517	15,679	15,703
	Eschweilera gigantea (Knuth) J. F. Macbr.	1	15,721					
	Eschweilera micrantha (Berg) Miers	1	15,724					
	Eschweilera rufifolia S. A. Mori	9	14,761	14,690	15,089	15,088	15,110	15,128
	Eschweilera tessmannii Knuth	2	15,102	15,134				
	Eschweilera sp.	1	14,638					
	Eschweilera sp.	1	14,661					
	Gustavia hexapetala (Aubl.) Sm.	1	15,160					
	Gustavia longifolia Poepp. ex Berg	2	14,625	14,628				
	Lecythis pisonis aff. Cambess.	1	15,097					
	Lecythis sp. cf. *chartacea* Berg	1	15,066					
Lepidobotryaceae	*Ruptiliocarpon* sp.	3	14,820	15,543	15,740			
Linaceae	*Hebepetalum humiriifolium* (Planch.) Benth.	1	15,018	15,126				
	Raucheria punctata (Ducke) Ducke	1	15,791 ft					
Loganiaceae	*Strychnos ramentifera* Ducke***	1	15,054					
	Strychnos sandwithiana Krukoff & Barneby***	1	15,775					
Malpighiaceae	*Byrsonima cowanii* W. R. Anderson	1	15,714					
	Brysonima poeppigiana A. Juss.	2	14,809	15,001				
	Byrsonima stipulina A. Juss.	8	14,902	14,927	15,031	15,049	15,431 fl	15,448 bd
	Dicella julianii (J. F. Macbr.) W. R. Anderson***	1	15,549					
	Tetrapterys mucronata Cav.***	1	15,669					
Marcgraviaceae	*Norantea guianensis* subsp. *japurensis* Aubl.***	1	14,892 fl					
Melastomataceae	*Miconia tetragona* Cogn.	1	14,969					

Appendix 1. Continued.

Family	Taxon and authority	No. plants in 2-ha	Vásquez collections (only the first six are listed per taxon)					
Meliaceae	*Carapa procera* DC.	6	14,629	14,647	14,653	14,679	14,863	14,891
	Guarea silvatica DC.	1	15,790					
	Guarea sp.	1	14,759					
	Guarea sp.	1	14,645					
	Trichilia sp. cf. *micrantha* Benth.	3	14,780	14,813	14,893			
Menispermaceae	*Abuta grandifolia* (Mart.) Sandwith	3	15,518	15,623	15,654			
	Abuta sp.***	1	15,264					
	Abuta sp. cf. *imene****	1	15,058					
	Curarea tecunarum Barneby & Krukoff***	1	15,101					
	Telitoxicum krukovii Moldenke***	1	15,277					
	Telitoxicum minutifolium (Diels) Moldenke***	1	15,466					
	Telitoxicum peruvianum Moldenke***	1	15,282					
Monimiaceae	*Siparuna cristata* (Peopp. & Endl.) A. DC.	1	15,527					
	Siparuna cuspidata (Tul.) A. DC.	1	15,781					
	Siparuna decipiens (Tul.) A. DC.	5	14,674 bd	14,766 bd	15,505	15,565	15,597 ft	
	Siparuna guianensis Aubl.	3	14,709	14,723	14,782			
Moraceae	genus cf. *Castilla*	1	15,203A					
	Brosimum acutifolium subsp. *obovatum* (Ducke) C. C. Berg	1	15,289					
	Brosimum guianense (Aubl.) Huber	3	15,487	15,616	15,709 bd			
	Borsimum lactescens (S. Moore) C. C. Berg	1	15,630					
	Brosimum potabile Ducke	1	14,741					
	Brosimum rubescens Taub.	3	14,854	14,964	15,127			
	Brosimum utile subsp. *ovatifolium* (Kunth) Pittier	5	14,836	15,165 ft	15,184	15,554	15,571	
	Castilla ulei Warb.	1	15,652					
	Cecropia distachya Huber	3	14,725 fl	15,021	15,288			
	Cecropia engleriana Snethl.	2	15,707 fl	15,708				
	Cecropia sciadophylla Mart.	1	15,615 fl					
	Ficus amazonica (Miq.) Miq.	1	15,163					
	Ficus subapiculata (Miq.) Miq.	1	15,626					
	Helicostylis tomentosa (Poepp. & Endl.) Rusby	4	14,637	14,778	15,130	15,191		
	Maquira calophylla (Poepp. & Endl.) C. C. Berg	3	14,705	15,609	15,695			

Appendix 1. Continued.

Family	Taxon and authority	No. plants in 2-ha	Vásquez collections (only the first six are listed per taxon)				
Moraceae	*Naucleopsis imitans* (Ducke) C. C. Berg	1	15,798				
	Naucleopsis mello-barretoi (Standl.) C. C. Berg	1	15,668				
	Naucleopsis ternstroemiiflora (Mildbr.) C. C. Berg	1	15,376				
	Naucleopsis ulei (Warb.) Ducke	1	15,716				
	Perebea glabrifolia C. C. Berg	2	14,791	15,170			
	Perebea guianensis Aubl. subsp. *guianensis*	4	14,733	14,735	14,740	15,735	
	Pourouma bicolor Mart. subsp. *bicolor*	1	15,189				
	Pourouma bicolor subsp. *scobina* (Benoist) C. C. Berg	1	15,068	15,189			
	Pourouma ferruginea Standl.	1	15,683				
	Pourouma guianensis Aubl. subsp. *guianensis*	2	14,805	15,726			
	Pourouma herrerensis C. C. Berg	1	15,085				
	Pourouma minor Benoist	1	15,532				
	Pourouma sp.	1	14,631				
	Pseudolmedia laevis (Ruiz & Pav.) J. F. Macbr.	3	14,602	14,685	15,732		
	Pseudolmedia laevigata Trécul	6	14,842	14,903 bd	14,916	14,946	15,309	15,481
	Trymatococcus amazonicus Poepp. & Endl.	2	14,698 ft	15,699			
Myristicaceae	*Campsoneura capitellata* (A. DC.) Warb.	1	15,809				
	Iryanthera crassifolia A. C. Sm.	4	15,070	15,082	15,193	15,808	
	Iryanthera elliptica Ducke	1	15,146				
	Iryanthera juruensis Warb.	13	14,618	14,640	14,712	14,748	14,800	15,503
	Iryanthera laevis Markgr.	3	14,745	14,978	15,546		
	Iryanthera lancifolia Ducke	4	14,639	14,803	15,040	15,780	
	Iryanthera macrophylla (Benth.) Warb.	7	14,706	14,760	14,889	15,330 ft	15,752	15,786
	Iryanthera paradoxa (Schwacke) Warb.	9	14,739	14,846	14,853	14,865	14,890	14,901
	Iryanthera paraensis Huber	3	14,668	15,067	15,656		
	Iryanthera polyneura Ducke	2	14,855	15,482			
	Iryanthera ulei Warb.	29	14,603	14,722	14,743	15,287 ft	15,291 ft	15,510 ft

Appendix 1. Continued.

Family	Taxon and authority	No. plants in 2-ha	Vásquez collections (only the first six are listed per taxon)					
Myristicaceae	*Iryanthera* sp.	1	15,262					
	Iryanthera sp.	1	15,519					
	Osteophloeum platyspermum (A. DC.) Warb.	4	14,701	15,484	15,561	15,750		
	Otoba glycicarpa W. Rodr.	2	15,560	15,641				
	Virola calophylla Warb.	4	15,050	15,149	15,419 bd	15,465 ft		
	Virola divergens Ducke	1	15,823					
	Virola duckei A. C. Sm.	1	15,591					
	Virola elongata (Benth.) Warb.	2	14,767 ft	15,098				
	Virola mollisima (A. DC.) Warb.	1	15,725					
	Virola multinervia Ducke	3	14,795	15,174	15,644			
	Virola pavonis (A. DC.) A. C. Smith	23	14,619	14,649	14,669	14,682	14,789	15,272 ft
Myrsinaceae	*Cybianthus peruvianus* Miq.	1	15,016					
Myrtaceae	genus	1	15,675					
	genus	1	15,687					
	genus	1	15,390					
	genus	1	15,769					
	genus	1	14,715					
	genus	1	14,816					
	genus	1	14,857					
	genus	1	14,995					
	genus	1	15,033					
	genus	2	15,408	15,410				
	genus	1	15,400					
	genus	1	15,471					
	genus	1	voucher lost					
	Calyptranthes crebra McVaugh	1	14,956					
	Calyptranthes cuspidata DC.	1	14,971					
	Calyptranthes simulata McVaugh	1	14,954					
	Eugenia patrisii Vahl	1	15,534					
	Marliera caudata McVaugh	3	15,266	15,343	15,443			
	Marliera imperfecta McVaugh	1	14,955					

Appendix 1. Continued.

Family	Taxon and authority	No. plants in 2-ha	Vásquez collections (only the first six are listed per taxon)					
Nyctaginaceae	genus	1	15,194 ft					
	genus	1	15,557					
	genus	1	14,696					
	genus	1	voucher lost					
	Neea divaricata Poepp. & Endl.	2	14,671	15,820				
	Neea floribunda Poepp. & Endl.	8	15,383	15,387	15,412	15,415	15,430	15,439
	Neea parviflora Poepp. & Endl.	1	15,795					
	Neea spruceana Heimerl	3	15,655	15,710	15,711			
	Neea verticellata Ruiz & Pav.	1	14,737					
	Neea sp. cf. *macrophylla* Poepp. & Endl.	1	15,403					
Ochnaceae	*Cespedesia spathulata* (Ruiz & Pav.) Planch.	1	14,911	14,912				
	Ouratea sp.	1	15,248					
Olacaceae	*Aptandra tubicina* (Poepp.) Benth. ex Miers	1	15,087 ft					
	Cathedra acuminata (Benth.) Miers	2	14,909 bd	15,192				
	Minquartia guianensis Aubl.	4	14,811	15,670	15,712	15,763		
	Schoepfia lucida Pulle	1	15,603					
Opiliaceae	*Agonandra brasiliensis* Benth.	1	15,737					
	Agonandra silvatica Ducke	1	15,530					
	Agonandra sp.	1	15,807					
Palmae	*Astrocaryum chambira* Burret	6	14,801 fl					
	Astrocaryum macrocalyx Burret	14	14,592					
	Euterpe precatoria Mart.	7	14,623	14,828 ft				
	Iriartea deltoidea Ruiz & Pav.	13	14,593 fl					
	Oenocarpus bataua Mart.	78	14,633	15,020				
	Scheelea moorei Glassman	3	no voucher					
	Socratea exorrhiza (Mart.) H. Wendl.	2	14,617 ft					
Polygonaceae	*Coccoloba* sp.***	1	14,960	15,613				
	Coccoloba sp.	1	15,613					
	Moutabea sp.***	1	15,550					
Quiinaceae	*Lacunaria jenmanii* (Oliver) Ducke	1	14,864					
	Quiina obovata Tul.	3	14,687	15,142	15,167			
	Quiina sp.	1	15,749					

Appendix 1. Continued.

Family	Taxon and authority	No. plants in 2-ha	Vásquez collections (only the first six are listed per taxon)				
Rhizophoraceae	*Cassipourea peruviana* Alston	1	15,601				
Rosaceae	*Prunus detrita* vel sp. aff. J. F. Macbr.	5	14,958	15,367	15,397	15,446	15,451
Rubiaceae	*Alseis microarpa* Standl. & Steyerm.	1	15,661				
	Borojoa clavifora (K. Schum.) Cuatrec.	2	15,180	15,636			
	Ferdinandusa chlorantha (Wedd.) Standl.	5	15,242	15,344	15,372	15,374	15,452
	Kotchubaea sericantha Standl.	2	14,999	15,743			
	Pagamea coriacea Spruce ex Benth.	1	15,423				
	Rudgea fissistipula Muell. Arg.	1	14,915 ft				
Rutaceae	*Zanthoxylum huberi* Waterman	1	15,090				
Sabiaceae	*Meliosma herbertii* Rolfe	2	15,283 ft	15,286			
	Ophiocaryon heterophyllum (Benth.) Urb.	2	15,497	15,541			
	Ophiocaryon klugii Barneby	3	15,610	15,646	15,696		
	Ophiocaryon manausense (W. A. Rodrigues) Barneby	1	14,978				
Sapindaceae	*Cupania diphylla* Vahl	1	15,111				
	Cupania sp.	1	14,697				
	Matayba arborescens (Aubl.) Radlk.	1	15,029 ft				
	Matayba guianensis Aubl.	1	15,828				
	Matayba sp.	3	14,935	15,000	15,023		
	Talisia japurensis Radlk.	1	15,175				
	Talisia sp.	1	15,132				
Sapotaceae	Genus cf. *Ecclinusa*	1	21,365				
	Chrysophyllum amazonicum T. D. Penn.	3	15,118	15,520	15,596		
	Chrysophyllum bombycinum T. D. Penn.	9	14,866	14,930	14,976	15,034	15,072	15,249
	Chrysophyllum cuneifolium (Rudge) A. DC.	2	15,137	15,202			
	Chrysophyllum sanguinolentum (Pierre) Baehni	4	14,814	14,848	14,997	15,053	
	Ecclinusa lanceolata (Mart. & Eichler) Pierre	4	14,807	15,012	15,326	15,328	
	Ecclinusa ramiflora Mart.	1	14,884				
	Micropholis acutangula (Ducke) Eyma	1	14,713				
	Micropholis casiquiarensis Aubrev.	4	14,675 fl	14,806	15,096	15,524	
	Micropholis egensis (A. DC.) Pierre	3	14,835 bd	14,883	15,133		

Appendix 1. Continued.

Family	Taxon and authority	No. plants in 2-ha	Vásquez collections (only the first six are listed per taxon)					
	Micropholis guyanensis subsp. *duckeana* (A. DC.) Pierre	4	14,938	14,986	15,409	15,590		
	Micropholis guyanensis (A. DC.) Pierre subsp. *guyanensis*	2	15,092	15,369				
	Micropholis venulosa (Mart. & Eichler) Pierre	1	15,052					
	Pouteria bangii (Rusby) T. D. Penn.	5	14,849	15,037	15,076	15,129	15,187	
	Pouteria caimito (Ruiz & Pav.) Radlk.	4	14,755	15,100	15,535	15,697		
	Pouteria cuspidata (A. DC.) Baehni	5	15,276	15,359	15,396	15,475	15,572	
	Pouteria eugeniifolia vel. sp. aff. (Pierre) Baehni	1	15,119					
	Pouteria multiflora vel sp. aff. (A. DC.) Eyma	2	14,727	15,086				
	Pouteria reticulata (Engl.) Eyma subsp. *reticulata*	1	15,723					
	Pouteria rostrata (Huber) Baehni	1	14,702					
	Pouteria subrotata Cronquist	1	14,781					
	Pouteria torta subsp. *glabra* T. D. Penn.	3	15,153	15,153	15,154	15,779		
	Pouteria torta subsp. *tuberculata* (Sleumer) T. D. Penn.	2	14,720 fl	14,746 fl				
	Pouteria sp.	1	14,790					
	Pouteria sp.	1	14,717					
Simaroubaceae	*Simaba guianensis* Aubl.	1	14,607					
	Simaba polyphylla (Cavalc.) W. Thomas	1	15,688					
	Simarouba amara Aubl.	6	14,606	14,952	15,178	15,495	15,576	15,677
Sterculiaceae	*Sterculia apeibophylla* Ducke	1	15,639					
	Sterculia pruriens (Aubl.) K. Schum.	3	14,686	15,172	15,529			
	Sterculia tessmannii Mildbraed	2	15,186	15,507				
	Sterculia sp.	1	15,144					
	Sterculia sp.	1	15,539					
	Theobroma glaucum H. Karst.	1	15,734					
	Theobroma obovatum Klotzsch ex Bernoulli	1	15,556					
	Theobroma subincanum Mart.	10	14,635	14,716	14,792	15,008	15,051	15,114

Appendix 1. Continued.

Family	Taxon and authority	No. plants in 2-ha	Vásquez collections (only the first six are listed per taxon)					
Tiliaceae	*Apeiba aspera* Aubl.	3	15,014 bd	14,752 bd	15,672 fl			
	Luehea sp.	1	14,714 fl					
	Luehea sp.	1	14,648					
	Luehea sp.	1	14,658					
	Luehea sp.	1	15,685					
	Luehea sp.	1	15,754					
Violaceae	*Leonia glycycarpa* Ruiz & Pav. var. *glycycarpa*	8	14,644	14,672	14,796	15,106	15,168	15,483
	Paypayrola sp.	1	15,205					
	Rinorea flavescens (Aubl.) Kuntze	1	15,190					
	Rinorea racemosa (Mart.) Kuntze	9	14,711	14,754	15,105	15,115	15,161 ft	15,200
	Rinorea sp.	1	15,796					
	Rinorea sp.	1	15,718 ft					
	Rinorea sp.	1	15,706					
Vochysiaceae	*Qualea paraensis* Ducke	2	15,784	15,829				
	Vochysia densifolia Spruce	1	14,666					

A SYSTEMATIC TREATMENT OF *ACACIA COULTERI* (FABACEAE, MIMOSOIDEAE) AND SIMILAR SPECIES IN THE NEW WORLD[1]

Jennifer T. Jawad,[2] David S. Seigler,[3] and John E. Ebinger[4]

ABSTRACT

Detailed descriptions, habitat preferences, geographic ranges, and representative specimens are given for the 13 taxa of the *Acacia coulteri* group from Mexico, Central America, and the southwestern United States. These species form a distinct group within *Acacia* series *Vulgares*, lacking prickles and usually having persistent stipules. A principal components analysis (PCA) of vegetative and floral features shows that the specimens examined form discrete units in plots of the first three principal components. The groups established by PCA mostly coincide with previously described species. The taxa within this group are phenetically similar, sharing many morphological features. These data also suggest that there is occasional gene flow between species, but that hybrids are not common. About half the species have restricted ranges (*A. compacta*, *A. dolichostachya*, *A. durangensis*, *A. millefolia*, *A. russelliana*, *A. sericea*, and *A. willardiana*), but the remainder are wide-ranging, either from Oaxaca and Puebla north into central and northern Mexico (*A. acatlensis*, *A. coulteri*, *A. mammifera*, and *A. salazari*), or south into Central America (*A. centralis* and *A. usumacintensis*).

Key words: *Acacia coulteri* group, *Acacia* series *Vulgares*, Fabaceae, phytogeography, principal components analysis, species delineation, hybridization.

Acacia Miller series *Vulgares* Benth. (Fabaceae: Mimosoideae), consists of about 200 species distributed in tropical and subtropical wet to dry habitats, in both the Old and New Worlds. Series *Vulgares* broadly corresponds to subgenus *Aculeiferum* Vassal (1972), according to Pedley (1978). Later, Pedley (1986) suggested that this subgenus should be elevated to generic status under the name *Senegalia* Raf. More recent systematic studies suggest that *Acacia* series *Vulgares* may not represent a monophyletic group (Chappill & Maslin, 1995; Clarke, 1995).

Most species of *Acacia* series *Vulgares* are of diverse habitat, habit, morphology, and chemistry. Most are shrubs or small trees, though some are tree-lianas, with branches scrambling over other vegetation. Most species are armed with prickles on the stems, leaf petioles, and rachises. Leaves are bipinnately compound with deciduous stipules, and numerous pairs of pinnae and leaflets. Peduncles are solitary, or in racemose clusters, from the leaf axils, and flowers form globose heads or cylindrical spikes. Legumes of series *Vulgares* are relatively large, strongly flattened, usually dehiscent, and with a single row of relatively large seeds. This series is often separated from two other *Acacia* series, *Gummiferae* and *Filicinae*, by the presence of prickles that are scattered along the stems and sometimes the leaf rachises (Bentham, 1875).

Acacia series *Vulgares* can be divided into several informal "species groups" based on overall similarities of habit, stipule persistence, the presence or absence of prickles, petiolar gland shape and structure, as well as inflorescence shape. At present, however, these informal groups within the series have not been established to be monophyletic units. The taxa of one of these groups of phenetically similar species, "the *Acacia coulteri*

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group" (Maslin & Stirton, 1997), are erect shrubs or small trees with persistent stipules and flowers in cylindrical spikes. Except for minor differences in flower size and occasionally pubescence, flowers are quite similar, with a tubular calyx and corolla, each 5-lobed, numerous separate stamens, and a short-stalked pistil. The 13 species of the *A. coulteri* group range from Arizona, south through Mexico into Costa Rica. They are morphologically distinct from other species of *Acacia* series *Vulgares* in that they always lack prickles, are never lianas, and by a number of other features. Nonetheless, taxonomic treatments have not dealt with this group as a separate unit within the *Acacia* series *Vulgares* (Bentham, 1875; Standley, 1920; Britton & Rose, 1928).

Many sterile and fruiting specimens of species of the *A. coulteri* group have been misidentified, adding to the taxonomic confusion. Flowering specimens of *Albizia*, *Lysiloma*, and *Piptadenia* can easily be distinguished from spicate species discussed in this work. In contrast to the *A. coulteri* group; *Albizia* and *Lysiloma* have capitate inflorescences. Although inflorescences of *Piptadenia* species are spicate, these flowers have 10 stamens with strap-like filaments and oblong anthers that are larger than those of *Acacia*. Stamen filaments of flowering specimens of *Albizia* and *Lysiloma* have stamens of about the same number and size as those in *Acacia*, but the filaments are united into a distinct tube. Capitate species are normally evident, even in fruiting condition. Most fruiting specimens of *Piptadenia* can be recognized by stamen remnants at the base of the fruits; its strongly venose fruits appear distinct from those of *Acacia* species, especially if comparative material is available. Careful search of most *Piptadenia* specimens reveals the presence of prickles, which are completely absent from all members of the *A. coulteri* group, as well as from members of the genera *Albizia* and *Lysiloma*. Two spicate species of the latter genus, *L. acapulcense* and *L. auritum*, are sometimes confused with members of the *A. coulteri* group. For these two species, the two parallel sutural ribs are fused around the periphery of the pod; these ribs often separate from the valves (Barneby & Grimes, 1996). Vegetative specimens of several *Lysiloma* species may be confused with *Acacia* species of this group. Some *Lysiloma* species have cordate-auriculate stipules (at least on young branches) that are never found on the species treated in this work. Finally, vegetative specimens of *Albizia* usually can be differentiated by the palmate/pinnate venation present in the leaflets (Barneby & Grimes, 1996).

The only other member of *Acacia* series *Vulgares* with which species of the *A. coulteri* group are easily confused is *A. macilenta* Rose. Often, herbarium specimens of this last species lack prickles, and those specimens that lack prickles are superficially similar to some members of the *A. coulteri* group. Their separation is straightforward, however, as *A. macilenta* has petioles that are consistently less than 25 mm long, and the single petiolar gland is dark brown and shallowly volcano-shaped. In large-leaved species of the *A. coulteri* group with which *A. macilenta* might be confused, the petioles exceed 25 mm in length and have petiolar glands that are light green or yellow and not volcano-shaped.

Concepts and identification of taxa of the *A. coulteri* group have been unclear in the past. No functional keys that permit accurate identification of these taxa have been published. Surprisingly, in most herbaria, up to half the specimens of this group of species were misidentified. Further, a cladistic analysis based on molecular data from chloroplast restriction site analyses (Clarke, 1995), and cladistic analyses by Catherine Glass and David S. Seigler based on morphological data (unpublished) suggest a more ancient origin of these taxa than other species of *Acacia* series *Vulgares*. Otherwise, there are no comprehensive studies on the phylogenetic relationships of the *Acacia coulteri* group. The present study was undertaken to clarify the taxonomic status and discreteness of the taxa involved, as well as to provide accurate descriptions of and functional keys to these species.

Materials and Methods

About 500 *Acacia* specimens, obtained from 27 herbaria (A, ARIZ, ASU, BM, BR, CM, DS, EIU, F, G, GH, ILL, ISC, LL, MEXU, MIN, MO, MU, NY, POM, RSA, SD, TEX, UC, US, VT, WIS), were studied to determine the geographic ranges and morphological variation of the species in the *A. coulteri* group. These specimens were initially separated into groups based on the similarity of morphological characteristics. After removal of duplicate and incomplete specimens, only 15 to 25 specimens of most groups remained available for subsequent analysis. Only unambiguous specimens were scored and are indicated in the exsiccatae by an asterisk. Of the original specimen pool, 237 were scored for 1 floral and 14 vegetative characters (Table 1). All characters were measured for each specimen (three or more measurements) and plotted to confirm that gaps existed in order to permit the use of scored characters. The data were then analyzed by principal components analysis (PCA) using NTSYS-pc version 1.70 (Rohlf, 1993).

Table 1. Characters scored for the principal component analysis of the 13 species of the *Acacia coulteri* group.

1. Bark of trunk (1 = not exfoliating, 2 = exfoliating).
2. Petiole pubescence (1 = glabrous or nearly so, 2 = puberulent with appressed hairs, 3 = woolly pubescent).
3. Petiole length (1 = 0–10 mm, 2 = >10–99 mm, 3 = >99 mm).
4. Small purple glands on petiole, rachis, and pinnae rachises (1 = absent, 2 = present).
5. Petiole gland structure (1 = sessile, 2 = stalked, 3 = absent).
6. Petiole gland apex (1 = flattened, 2 = doughnut-shaped, 3 = globose, 4 = absent).
7. Rachis length (1 = absent, 2 = 1–20 mm, 3 = >20 mm).
8. Number of pinna pairs (1 = 1 to 6, 2 = 7 or more).
9. Petiolule length (1 = ≤2.4 mm, 2 = >2.4 mm).
10. Distance between leaflets (1 = ≤1.5 mm, 2 = >1.5 mm).
11. Number of leaflet pairs per pinna (1 = <36, 2 = >36).
12. Leaflet length (1 = 4–36 mm, 2 = >36–65 mm).
13. Leaflet apex (1 = obtuse to broadly acute, 2 = narrowly acute to acuminate).
14. Leaflet pubescence (1 = glabrous or nearly so, 2 = pubescent with appressed hairs beneath, 3 = pubescent with appressed hairs on both surfaces, 4 = long hairs clustered at the base of midrib beneath).
15. Perianth pubescence (1 = glabrous or nearly so, 2 = pubescent with appressed hairs, 3 = woolly pubescent).

Data were transformed by a square root transformation (Sokal & Rohlf, 1969). Groups were analyzed separately, in various combinations, and simultaneously.

Because cyanogenesis has previously been found in many *Acacia* species, and is sometimes useful taxonomically (Seigler & Ebinger, 1988, 1995; Clarke et al. 1989, 1990), all specimens were tested for the presence of cyanogenic compounds by the Feigl-Anger method (Feigl & Anger, 1966; Tantisewie et al., 1969). Seigler et al. (1978) reported a few herbarium specimens of *A. acatlensis* that released cyanide, and Conn et al. (1989) reported weakly positive cyanide tests for both *A. acatlensis* and *A. coulteri* using fresh, air-dried leaf material. During the present study, no material examined gave a positive-test for cyanide, even after the addition of emulsin.

Results and Discussion

When the entire set of 237 specimens was analyzed using PCA, the first three principal components accounted for 28%, 18%, and 17% respectively, or 63% of total variance (Tables 1, 2). The amount of variance contributed by the remaining components diminished slowly from the third principal component onward. The best perspective for display of the phenetic groupings was seen in the ordination based on the second and third principal components (Fig. 1). This figure presents all taxa, clearly resolved in one view. In this plot, 13 distinct groups, corresponding to the 13 previously defined species can be observed, although the data clusters of some groups are proximal. Perianth pubescence, petiolule length, and exfoliating bark (characters 15, 9, 1) were the most important characters for determining the component score for the first principal component axis (Table 1). Petiolar gland apex, petiolar gland structure, and the number of pinna pairs (characters 6, 5, 8) were most important for the second principal component axis. The most important characters for the third principal component axis were leaflet pairs per pinna, petiole pubescence, and the distance between leaflets (characters 11, 2, 10).

Table 2. Principal components for the data set for the *Acacia coulteri* group.

Character number	Component number		
	1	2	3
1	0.704	0.113	0.203
2	−0.638	−0.033	0.678
3	0.698	−0.244	0.008
4	−0.651	−0.040	0.526
5	0.396	0.782	0.250
6	0.352	0.810	0.435
7	−0.446	0.428	−0.272
8	−0.139	0.564	−0.409
9	0.744	−0.144	0.024
10	0.427	−0.444	0.589
11	−0.280	−0.189	−0.761
12	0.356	−0.368	0.353
13	0.469	0.545	−0.188
14	−0.404	0.394	0.441
15	−0.773	0.006	0.189
Percent of variance	28.2	17.9	17.2

The clusters on this plot do not contain recognizable subgroups, and, except for *A. usumacintensis* and *A. dolichostachya*, which nearly overlap on this plot, each of the clusters is clearly separated from the others (Fig. 1). In addition, the OTUs of each group are closely spaced, indicating that the species are homogeneous.

In addition to those herbarium specimens borrowed, the present treatment is also based on extensive collections and observations of these taxa

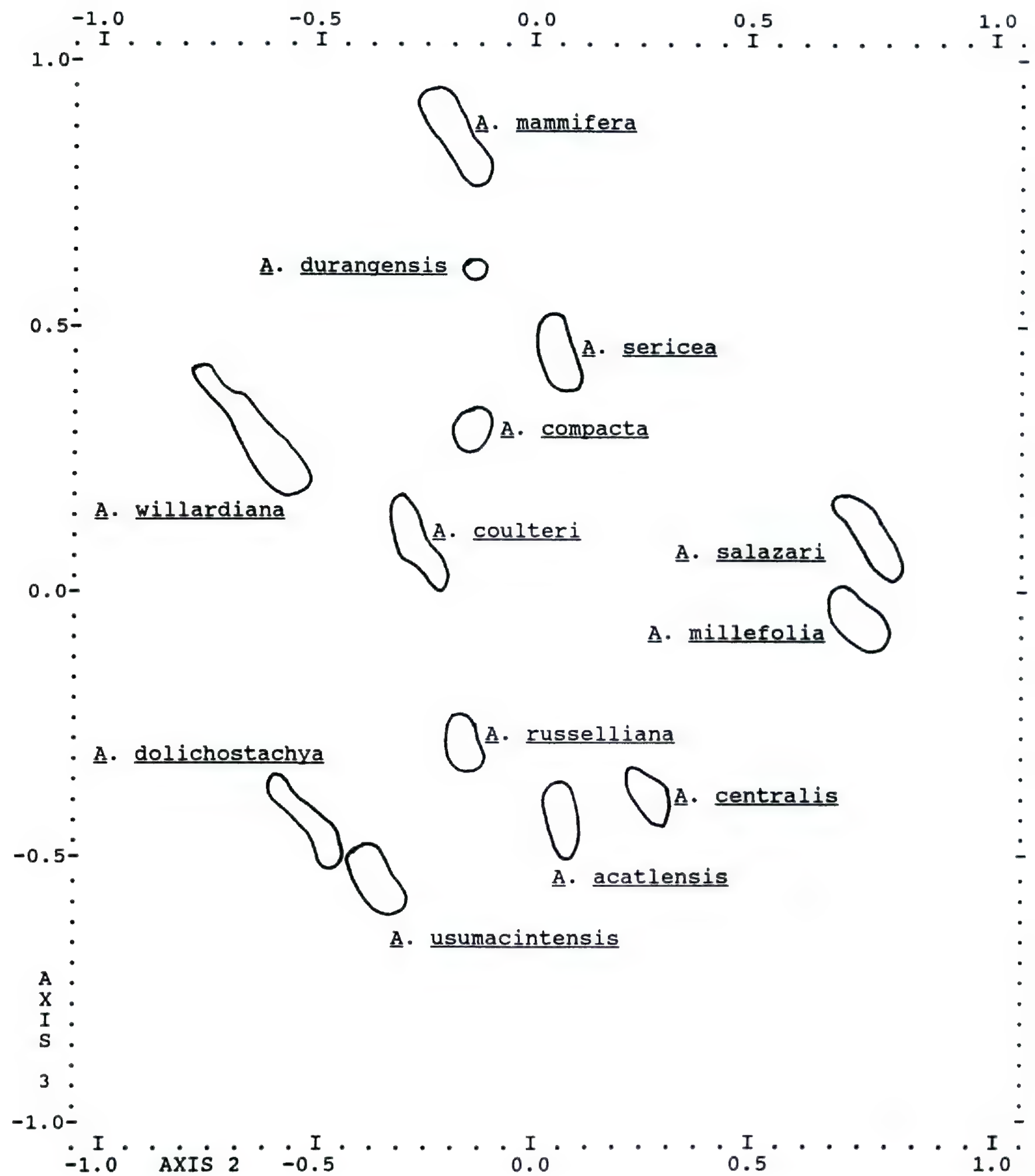

Figure 1. Plot of axis 2 versus 3 of a principal components analysis, using one floral and 14 vegetative variables from 237 specimens of the *Acacia coulteri* group.

in Mexico and Central America by one of the authors (DSS) on 15 trips during 1975–1998. Only the most reliable, diagnostic characteristics were used to distinguish the taxa. Although floral characteristics were measured carefully, and are included in the descriptions, flower variation and character overlap precludes using them for distinguishing species. Extensive lists of representative specimens are included, as specimens are commonly misidentified. Also, all specimens used in the PCAs are included in the list of representative specimens (designated by an asterisk after the herbarium abbreviation). Synonyms and types are included, along with maps showing the geographic distribution of each species (Figs. 2, 3).

Occasionally, a few flowering specimens lack leaves, or have extremely small leaves, making it difficult to determine the number of leaflets per pinna and the number of pinnae per leaf. Even with these specimens, the number of pinnae per leaf of-

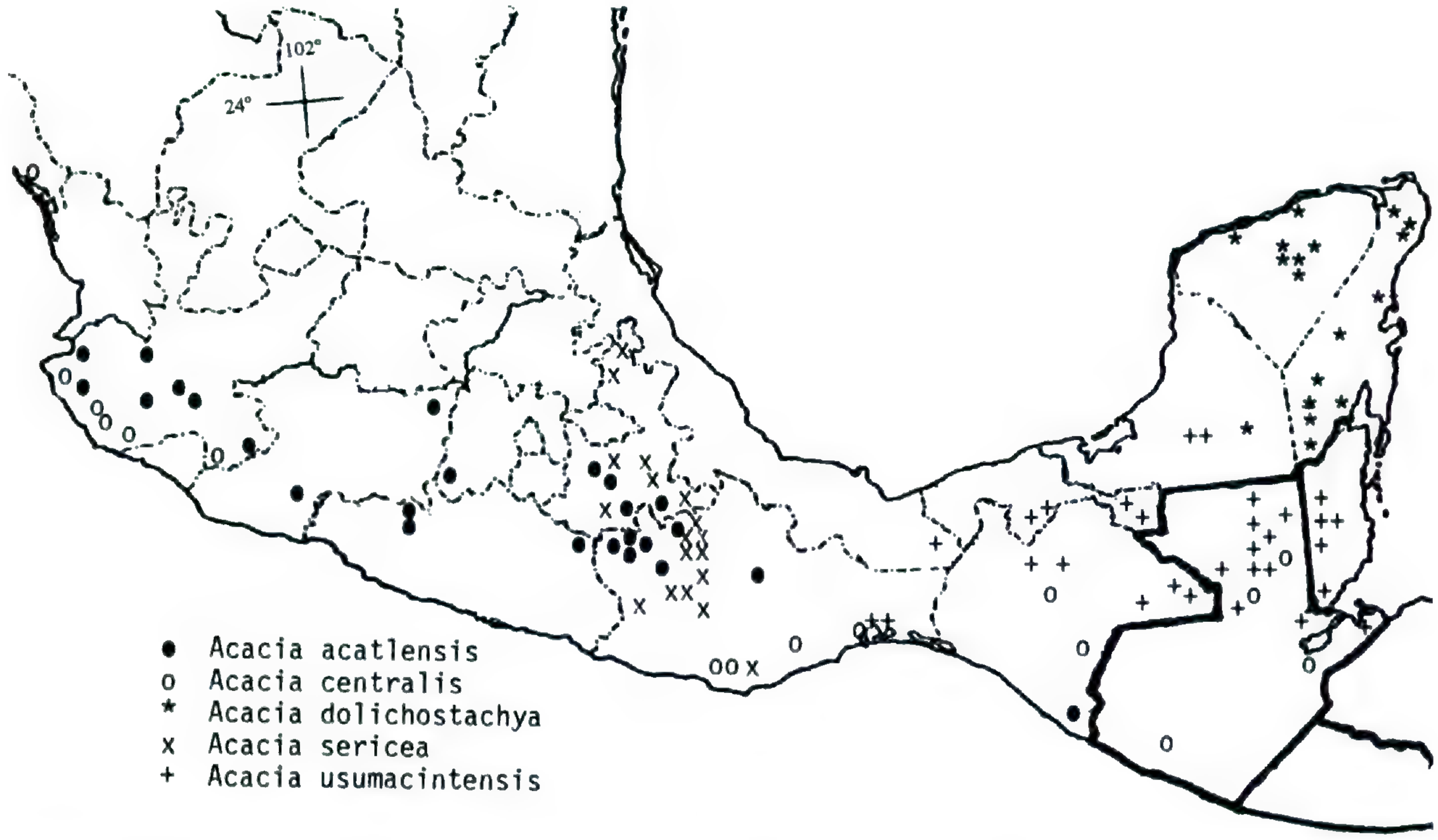

Figure 2. Distribution of *Acacia acatlensis*, *A. centralis*, *A. dolichostachya*, *A. sericea*, and *A. usumacintensis* in Mexico, Belize, and Guatemala. The distribution of *A. centralis* in the rest of Central America is not shown.

ten can be determined from small emerging leaves, or by counting the number of pinna scars on an old rachis that may be present. In excess of 500 specimens were examined in this study and few lacked leaves altogether. Most of these were either *Acacia acatlensis* or *A. sericea*. Minute purple glands on the young stems and peduncles of both taxa distinguish them from other members of this group of species. *Acacia acatlensis* is then separated from *A. sericea* because the former's calyx and floral bracts are only lightly appressed pubescent, whereas, in the latter, the calyx, corolla, and floral bracts are densely sericeous pubescent. Two other species have these small purple glands and occur within the same geographic range. *Acacia compacta* is a small shrub with short shoots, and *A. mammifera* is a small tree with flowers that have cream-colored corollas with a purplish tinge. However, these are only uncommonly found in flowering condition without leaves.

Key to Species

1a. Pinnae mostly with more than 36 pairs of leaflets, especially those near the middle of the rachis.
  2a. Petiolar gland(s) flattened, usually located on the lower third of the petiole; leaflet apex obtuse to broadly acute.
    3a. Leaflets 1.2–1.9 mm wide; leaves with more than 6 pairs of pinnae ..... 1. *A. usumacintensis*
    3b. Leaflets mostly less than 1.2 mm wide; most leaves with 6 or fewer pairs of pinnae ..... 2. *A. dolichostachya*
  2b. Petiolar gland(s) saucer-shaped to cup-shaped, usually located on the upper half of the petiole, rarely absent; leaflet apex narrowly acute to acuminate.
    4a. Minute purple glands common at the base of the leaflets and usually along the rachis; leaflets lacking long hairs on the lower side at the base ..... 3. *A. acatlensis*
    4b. Minute purple glands absent; leaflets usually with long hairs on the lower side at the base ..... 4. *A. centralis*
1b. Pinnae mostly with fewer than 36 pairs of leaflets, or pinnae absent.
  5a. Leaves less than 30 mm long, some clustered on short shoots ..... 5. *A. compacta*
  5b. Leaves more than 30 mm long; short shoots absent; pinnae sometimes absent in *Acacia willardiana*.
    6a. Leaflets appressed to erect pubescent on both surfaces, usually densely so; minute purple glands common on the rachis and pinna rachises.
      7a. Petiole and rachis densely pubescent with erect hairs about 0.3 mm long; petiolules less than 2.1 mm long; fruit pubescent ..... 6. *A. sericea*
      7b. Petiole and rachis glabrous or with short, appressed hairs; petiolules more than 2.0 mm long; fruit glabrous.

Figure 3. Distribution of *Acacia compacta, A. coulteri, A. durangensis, A. mammifera, A. millefolia, A. russelliana, A. salazari,* and *A. williardiana* in the U.S.A., Mexico, Belize, and Guatemala.

8a. Petiolar glands raised, the apex bulbous; most leaves with fewer than 7 pairs of pinnae ..... 7. *A. mammifera*

8b. Petiolar glands sessile and with an irregularly raised apex; most leaves with more than 10 pairs of pinnae ..... 8. *A. durangensis*

6b. Leaflets glabrous or lightly appressed pubescent beneath; minute purple glands absent.

9a. Leaves mostly with a single pair of pinnae (rarely 2 or 3); many petioles more than 100 mm long ..... 9. *A. willardiana*

9b. Leaves mostly with 4 or more pairs of pinnae; petioles less than 70 mm long.

10a. Rachis gland between the upper pinna pair stalked; shrub or small tree less than 4 m tall ..... 10. *A. millefolia*

10b. Rachis gland between the upper pinna pair sessile, usually saucer-shaped, cup-shaped, or absent; large shrub or tree; more than 4 m tall.

11a. Bark of trunk and larger branches exfoliating and papery; petiolar glands absent on many petioles; leaflet apex acuminate ..... 11. *A. salazari*

11b. Bark of trunk and larger branches smooth to furrowed, not exfoliating; petiolar glands present; leaflet apex broadly acute to obtuse.

12a. Leaflets appressed pubescent beneath; rachis and pinna rachises puberulent above; perianth pubescent ..... 12. *A. coulteri*

12b. Leaflets glabrous beneath; rachis and pinna rachises as well as perianth glabrous or nearly so ..... 13. *A. russelliana*

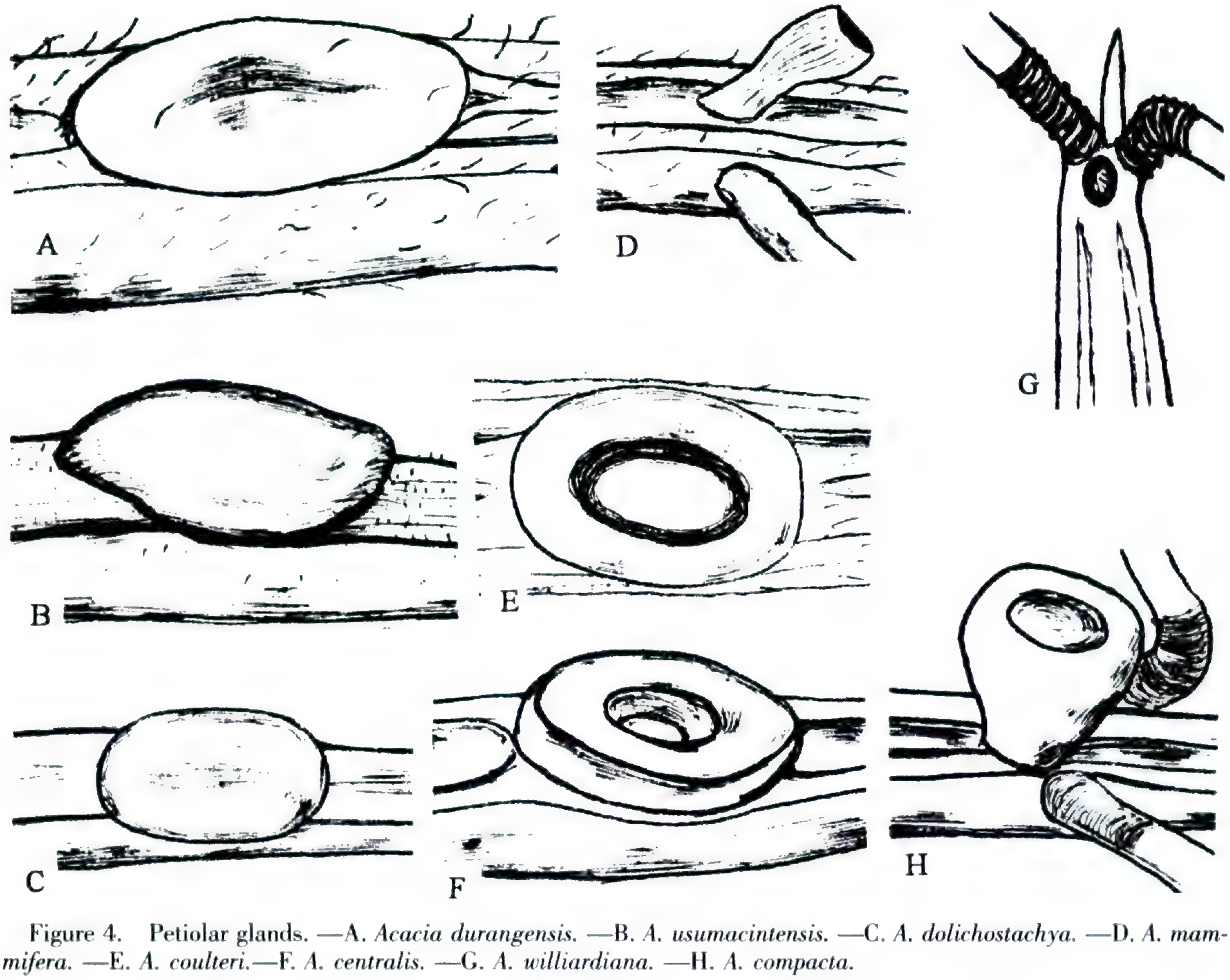

Figure 4. Petiolar glands. —A. *Acacia durangensis.* —B. *A. usumacintensis.* —C. *A. dolichostachya.* —D. *A. mammifera.* —E. *A. coulteri.*—F. *A. centralis.* —G. *A. williardiana.* —H. *A. compacta.*

1. Acacia usumacintensis Lundell, Contr. Univ. Michigan Herb. 4: 8. 1940. TYPE: Mexico. Tabasco: Boca Cerro on the Río Usumacinta above Tenosique, 1–5 July 1939, *E. Matuda 3550* (holotype, MICH!; isotypes, K!, LL!, MEXU!, MICH!, NY!).

Tree to 30 m tall with bark dark gray, shallowly furrowed; twigs brown to greenish brown, not flexuous, glabrous to lightly puberulent; short shoots absent. Leaves alternate, 90–200 mm long; stipules herbaceous, light brown, narrowly triangular, to 2.2 × 0.5 mm near the base, glabrous to lightly puberulent, persistent; petiole adaxially shallowly grooved, 30–70 mm long, glabrous to lightly puberulent; petiolar gland solitary, usually located on the lower third of the petiole, sessile, commonly elliptical, 1–6 mm long, flattened, glabrous (Fig. 4B); rachis adaxially grooved, 50–140 mm long, glabrous to lightly puberulent, a sessile, saucer-shaped gland, 1.0–1.9 mm across, between the upper pinna pair; pinnae 7 to 15 pairs per leaf, 55–90 mm long, 5–12 mm between pinna pairs; petiolules 1.7–3.0 mm long; leaflets (33)36 to 55 pairs per pinna, opposite, 1.0–1.6 mm between leaflets, oblong, 4–7 × 1.2–1.9 mm, glabrous, lateral veins obvious, with a midvein and 1 to 3 smaller veins from the base, base oblique, margins ciliate, apex acute to obtuse. Inflorescence a loosely flowered cylindrical spike 50–110 mm long, 1 to 3 from the leaf axil, or sometimes in terminal racemose clusters; peduncle 7–15 × 0.7–1.1 mm, puberulent; involucre absent; floral bracts linear, to 1 mm long, puberulent, early deciduous. Flowers sessile, creamy-white; calyx 5-lobed, 1.1–1.5 mm long, densely appressed pubescent; corolla 5-lobed, 1.8–2.5 mm long, densely appressed pubescent; stamen filaments 4–6 mm long; ovary glabrous to lightly pubescent, on a stipe to 0.3 mm long. Legumes light yellowish brown to dark brown, straight, flattened, oblong, 90–250 × 20–33 mm, cartilaginous, transversely striate, glabrous, eglandular, dehiscent; stipe to 22 mm long; apex acute. Seeds uniseriate, no pulp, dark reddish brown, oblong to oval, strongly flattened, 8.8–10.0 × 7.0–9.0 mm, smooth; pleurogram U-shaped, 3–4 mm across. Flowers: April–June.

Distribution. Moist tropical forests, along streams, and moist disturbed sites below 500 m

elevations in Belize, Guatemala, and the states of Campeche, Chiapas, Oaxaca, Tabasco, and Veracruz, Mexico (Fig. 2).

Acacia usumacintensis is a tall tree that is a common component of lowland tropical rainforests in Guatemala and southern Mexico. No specimens of this species have been observed by the present authors farther south than Guatemala and Belize. Specimens labeled *A. usumacintensis* from Nicaragua are the result of confusing this taxon with various species of *Lysiloma*. *Acacia usumacintensis* specimens are commonly misidentified as *L. acapulcensis* (Kunth) Benth. Thompson (1980), in a revision of *Lysiloma*, annotated the type of *A. usumacintensis* as *L. acapulcensis*. This specimen (*E. Matuda 3550*), however, is *A. usumacintensis*, having numerous separate filaments remaining at the base of some of the fruits.

Representative specimens. (*used in PCA analysis) BELIZE. El Cayo Dist., Benque Viejo, Río Mopán, *Contreras 7089* (CAS, F, LL, MO*, NY); vicinity Georgeville, Western hwy., *Dwyer 12662* (MO, US*); Valentin, El Cayo, *Lundell 6356* (F, GH, MICH*). GUATEMALA. **Izabal:** Puerto Barrios, ca. 20 km from town on the road to Machacas, *Marshall, Castillo & Marshall 423* (MO, NY*). **Petén:** Lacandón, about 2 km S, *Contreras 3467* (CAS, F, LL*, MO); Macanche, *Contreras 5479* (F, MICH*, US); Dos Lagunas, 19 Apr. 1969, *Contreras 8356* (F*, LL, MO); Tikal, in ramonal on Main Plaza, *Lundell 15348* (CAS*, F, LL, MO, NY); Tikal, around Aguada Tikal, *Lundell 15973* (F*, LL, NY); Sayaxche, in corozal about 5 km S of village, *Lundell 18071* (F, LL, MO, NY*); Cadenas, on river bank bordering Río Gracios, *Lundell & Contreras 19033* (F*, LL, NY). MEXICO. **Campeche:** El tormento Escárcega, *Sousa, Cabrera, Davidse & Chater 12369* (ILL, MO*). **Chiapas:** W side of Laguna Miramar E of San Quintín, *Breedlove 33387* (DS*, MICH, MO); 20 km al SE de la desviación San Javier Frontera Echeverría hacia Río Lacantum, *Chimal, Durán & Quintanilla 755* (F, ILL, MO*); a 5 km al E de Crucero Corozal, camino a Frontera Corozal, Ocosingo, *Martínez 7291* (MO, NY*); a 15 km al E de Nvo. San Juan Chamula camino a Ixcan, *Martínez & Girón 6288* (MO*, MU). **Oaxaca:** Rincón Bamba, al O de Salina Cruz, *Martínez 629* (MO*). **Tabasco:** Mun. Balancán, por la carretera O, por el Poblado Arroyo del Triunfo, *Calzada 2385* (F*, NY); La Palma, Balancán, *Matuda 3324* (F, MICH*, WIS). **Veracruz:** Mpio. Minatitlán, Lomas al SW de La Garganta, 5.8 km al W de Uxpanapa, *Wendt, Villalobos & Navarrete 4127* (F, MICH, MO, TEX).

2. Acacia dolichostachya S. F. Blake, Proc. Biol. Soc. Washington 34: 43. 1921. *Senegalia dolichostachya* (S. F. Blake) Britton & Rose, N. Amer. Fl. 23: 112. 1928. TYPE: Mexico. Yucatán: Las Bocas de Silam, May 1916, *G. J. Gaumer & Sons 23329* (holotype, F!; isotypes, F!, G!, K!, NY photo!).

Small tree to 15 m tall with bark dark brown to dark gray, scaly in rectangular plates; twigs light brown to greenish brown, not flexuous, glabrous; short shoots absent. Leaves alternate, 40–120 mm long, stipules herbaceous, light brown, narrowly triangular, to 1.3 × 0.3 mm near the base, glabrous, persistent; petiole adaxially shallowly grooved, 30–80 mm long, glabrous; petiolar gland solitary, located on the lower half of the petiole, sessile, circular to more commonly elliptic, 1.0–2.5 mm long, flattened or with slightly raised margins, glabrous (Fig. 4C); rachis adaxially grooved, 20–62 mm long, lightly puberulent, a sessile, saucer-shaped gland, 0.6–1.5 mm across, between the pinnae of the upper 1 to 2 pinna pairs; pinnae 2 to 6(8) pairs per leaf, 40–75(90) mm long, 4–9 mm between pinna pairs; petiolules 2.5–4.0 mm long; leaflets 36 to 65 pairs per pinna, opposite, 0.7–1.1 mm between leaflets, oblong, 3.5–5.5(7.5) × 0.8–1.3 mm, glabrous, lateral veins usually not obvious, only one vein from base, base oblique, margins ciliate, apex acute to obtuse. Inflorescence a loosely flowered cylindrical spike 20–90 mm long, 1 to 3 from the leaf axil, or rarely in terminal racemose clusters; peduncle 3–10 × 0.5–0.9 mm, glabrous to lightly puberulent; involucre absent; floral bracts linear, to 1 mm long, glabrous to lightly pubescent, usually not deciduous. Flowers sessile, creamy-white; calyx 5-lobed, 0.5–1.0 mm long, lightly appressed pubescent; corolla 5-lobed, 1.2–2.2 mm long, lightly appressed pubescent; stamen filaments 3–5 mm long; ovary glabrous, on a stipe to 0.3 mm long. Legumes light yellowish green to light brown, straight, flattened, oblong, 80–130 × 13–20 mm, cartilaginous, transversely striate, glabrous, eglandular, dehiscent; stipe to 12 mm long, apex acute to acuminate. Seeds not seen. Flowers: April–July.

Distribution. Common in thorn-scrub thickets, and disturbed dry forests in the lowlands of the states of Campeche, Quintana Roo, and Yucatán (Fig. 2).

Restricted to the Yucatán Peninsula, *Acacia dolichostachya* appears to be a relatively common component of thorn-scrub thickets and disturbed sites. The fact that most leaves have fewer than seven pinna pairs separates *A. dolichostachya* from most other species of this group. Other species with six or fewer pinna pairs differ by having pinnae with fewer than 35 pairs of leaflets, along with smaller leaflets (*A. compacta*), much longer petioles (*A. willardiana*), or pubescent leaves (*A. mammifera* and *A. sericea*).

Considering the restricted distribution of this species, the only other taxon that *A. dolichostachya* might be confused with is *A. usumacintensis*. Both taxa have more than 35 pairs of leaflets on each

pinna, flattened petiolar glands on the lower half of the petiole, and petiolules that are more than 2.5 mm long. They are easily separated as *A. dolichostachya* has fewer pinna pairs per leaf (mostly six or fewer), smaller stipules (less than 1.3 mm long), less distance between the leaflets on the pinna rachis (less than 1.2 mm), and smaller sepals (mostly less than 1.0 mm long).

Representative specimens. MEXICO. **Campeche:** 4 km despues de Sho laguna Xmaben, Mpio. Hopelchen, *Chan & Ucan 1023* (UC*); a 12 km al E de Constitución, o sea a 15 km al W de Conhuas, *Sousa, Ramamoorthy, Ibarra, Rico & Basurto 12229* (MO*). **Quintana Roo:** En Rancho San Felipe, a 7 km al S de la desviación a Puerto Morelos, por la carretera Cancún–Tulum, *Cabrera 11456* (MO*); a 15 km al N de la desviación a Puerto Morelos, sobre la carretera Tulum–Cancún, *Cabrera & Cabrera 3158* (MO*); a 12 km al E de Carrillo Puerto, rumbo a Vigia Chico, *Cabrera & Cabrera 3561* (MO*); a 35 km al NW de Carrillo Puerto, sobre el camino a Vigia Chico, *Cabrera & Cabrera 4080* (MO*); en el km 2 del camino al Faro de la Punta Celarain, Isla de Cosumel, *Cabrera & Cabrera 10555* (MO*); Jardín Botánico Quintana Roo, *Duran, Olmsted & Honorato 85* (MO*); 3–4 mi. S of Filipe Carrillo Puerto, *Dwyer, Spellman, Vaughan & Wunderlin 258* (MO*); 5 km al N de Puerto Morelos, *Grether, Quero & Flores 1331* (MO*); a 6 km al N de Puerto de Morelos a Cancún, *Sousa, Téllez & Cabrera 10959* (MO*); a 48 km al NE de Filipe Carrillo Puerto, *Sousa, Cabrera, Davidse & Chater 12441* (MO*); al NW del entronque Chetumal–F. Carrillo Puerto, sobre carretera Mérida via Corta, *Téllez & Cabrera 2012* (MO*); a 10 km al S de Tulum, *Téllez & Cabrera 2032* (MO, NY*); a 15 km al N de Bacalar, *Téllez & Cabrera 2366* (NY*); 10 km antes de la entrada a Buenavista, de Chetumal rumbo a Felipe Carrillo Puerto, *Ucan & Flores 985* (UC*). **Yucatán:** 11.2 km N of Dzibilchaltum turn on road from Mérida to Progreso, *Bradburn & Darwin 1294* (MO, NY*); 4.6 km al E de Sucila, sobre la carretera Tizimin–Mérida, *Cabrera & Cabrera 13203* (MO*); near Piste, *Lundell & Lundell 7344* (A, F, LL, MICH*, MO, US); 7 km al S de Yaxcaba, *Vara, Arias & Isely 574* (CAS, F*, MO).

3. Acacia acatlensis Bentham, London J. Bot. 1: 513. 1842. *Senegalia acatlensis* (Bentham) Britton & Rose, N. Amer. Fl. 23: 112. 1928. TYPE: Mexico. Puebla: Acatlán, May 1830, *G. Andrieux 396* (holotype, K!, F photo!, MICH photo!, NY photo!, TEX photo!; isotypes, G!, US!).

Senegalia submontana Britton & Rose, N. Amer. Fl. 23: 113. 1928. TYPE: Mexico. Oaxaca: Cerro San Antonio, alt. 1800 m, 12 Oct. 1907, *C. Conzatti 2046* (holotype, US!; isotypes, F!, MEXU!, NY!). [Britton and Rose (1928) listed the type as *Conzatti 25,346.* On the NY isotype, Britton wrote "the number of this specimen was erroneously printed 25,346."]

Shrub or small tree to 15 m tall with bark dark gray, shallowly furrowed; twigs light brown to greenish brown, not flexuous, glabrous to lightly puberulent; short shoots absent. Leaves alternate, 50–150 mm long; stipules herbaceous, light brown, narrowly linear, to 3 × 0.5 mm near the base, usually glabrous, persistent; petiole adaxially shallowly grooved, 15–40 mm long, ± glabrous, but commonly with minute purple glands; petiolar gland solitary, located between the lower pinna pairs or along the upper half of the petiole, sessile, nearly circular to slightly elongated, 0.7–2.3 mm long, saucer- to cup-shaped, glabrous, rarely absent; rachis adaxially grooved, 20–110 mm long, glabrous to lightly puberulent and usually with minute purple glands, a sessile, cup-shaped gland, 0.4–0.9 mm across, between the upper pinna pair; pinnae 7 to 30 pairs per leaf, 25–60 mm long, 3–7 mm between pinna pairs; petiolules 1.0–2.4 mm long; leaflets 36 to 60 pairs per pinna, opposite, 0.5–1.2 mm between leaflets, linear, 2.5–4.8 × 0.7–1.1 mm, glabrous and commonly light greenish purple above, lateral veins obvious, a midvein and 1 to 2 smaller veins from the base, base oblique, margins ciliate, apex narrowly acute to acuminate. Inflorescence a loosely flowered cylindrical spike 40–100 mm long, 1 to 4 from the leaf axil; peduncle 5–10 × 0.5–1.0 mm, glabrous to lightly puberulent and commonly with scattered minute purple glands; involucre absent; floral bracts linear, to 1 mm long, early deciduous. Flowers sessile, creamy-white; calyx 5-lobed, 1.0–1.6 mm long, lightly appressed pubescent; corolla 5-lobed, 2.1–2.8 mm long, lightly appressed pubescent; stamen filaments 4.5–6.5 mm long; ovary glabrous, on a stipe to 0.4 mm long. Legumes light to dark brown, straight, flattened, oblong, 80–170 × 13–25 mm, cartilaginous, transversely striate, glabrous, eglandular, dehiscent; stipe to 20 mm long; apex broadly acute. Seeds uniseriate, no pulp, dark reddish brown, oval, strongly flattened, 7–10 × 4.5–7.2 mm, smooth; pleurogram U-shaped, 1.2–2.3 mm across. Flowers: March–June.

Distribution. Dry, deciduous, tropical forests and thorn-scrub forests between 500 and 2100 m elevations in the states of Chiapas, Guerrero, Jalisco, México, Michoacán, Oaxaca, and Puebla, Mexico (Fig. 2).

Acacia acatlensis is very similar morphologically to *A. centralis.* Both are widely distributed in southern Mexico, although *A. centralis* has been collected more frequently in the very south of Mexico and, unlike *A. acatlensis,* it occurs across the border in Guatemala and countries to the south (see Fig. 2). These two species are difficult to separate, and it is possible that they should be considered as subspecies of a single species. The two can be sepa-

rated based on the presence or absence of minute purple glands that are common at the base of the leaflets, in the grooves of the rachis and petiole, and not uncommon along the axis of the inflorescence in *A. acatlensis*. Rarely these glands are clear, lacking the purple color, but are easily observed under magnification. None of these minute glands were found on specimens of *A. centralis* examined. No other characteristics could be found that consistently separate these two species, though most specimens of *A. centralis* have leaflets with long hairs at the base on the ventral surface. These hairs are particularly evident on young leaflets, sometimes falling off as the leaves mature.

Acacia acatlensis is also similar to *A. usumacintensis*, differing by having narrower leaflets (less than 1.1 mm across) and lacking the large flattened petiolar gland of that taxon. *Acacia acatlensis* is sympatric with *A. sericea* and *A. mammifera* in Oaxaca; these two taxa also have minute purple glands on the leaves. *Acacia acatlensis* is easily separated from *A. mammifera*, which has fewer than 33 pairs of leaflets per pinna, leaflets that are commonly more than 1.5 mm wide, and a stalked petiolar gland. The dense, erect pubescence of *A. sericea* petioles and fruits usually separates this species from *A. acatlensis*.

Representative specimens. MEXICO. **Chiapas:** a 4 km al SW de Toliman, *Sousa, Ramamoorthy, Cortés & Hernández 11824* (MO*, TEX). **Guerrero:** Tario, Coyuca Dist, *Hinton 7755* (DS, GH, MICH, NY, US); en el Puerto El Salado, a 7 km al N de Tlapa camino a Huamuxtitlán, *Martínez 1070* (MO, TEX*). **Jalisco:** 2 km al W de El Mirador, 10 km al NE de El Corcobado, carretera Autlán–Guadalajara, *Magallanes 2169* (MO*); a 20 km al NW de San Patricio, *Magallanes 2420* (MO*); 20 km NE of Autlán, *McVaugh 23271* (MICH*, MO, NY); Barranca de Los Tanques, desviación al camino San Martín de las Cañas, *Villarreal 6121* (MICH*). **México:** Pungarancho, Temascaltepec Dist., *Hinton 3747* (F, GH*, MO, NY, US). **Michoacán:** 3 km al S de Jungapeo, *Soto, Aureolea, Silva & Pizarras 8573* (MEXU*). **Oaxaca:** 5 km sobre la desviación a Barranca de Los Calabazos, *Aguilar 220* (NY*); 3 km al NE de Tonala, carretera a Huajuapán, *Calzada & Campos 18320* (CAS*, MICH, MSC); cave area near Mitla, near pass to Díaz Ordaz, *Kirkby 10* (US*); 2.5 km al N de Yosocuta en la carretera Huajuapán–Juxtlahuaca, *Magallanes, Ramos & Magallanes 11* (CAS*, LL, MO); 3 km al NW de Santo Domingo Tonala, carretera a Huajuapán, *Ortíz-Bermudez 291* (MEXU*); Tomellín Canyon, *Pringle 5885* (GH, MICH, US*, VT); cerca de Tonala, *Rzedowski 34920* (CAS*, MO); Subida a las Ruinas del Cerro Guiengola, *Torres, Torres, Cortéz & Martínez, 928* (MO*); Ladera Oriente del Cerro Guiengola, *Torres, Torres, Téllez & Martínez 400* (MO*). **Puebla:** Tepeji de Rodríguez, *Felger 85-38* (MO*); 29 mi. SE of Acatlán on hwy. 190, *Seigler & Maslin 12692* (EIU*, ILL).

4. Acacia centralis (Britton & Rose) Lundell, Contr. Univ. Michigan Herb. 4: 7. 1940. *Senegalia centralis* Britton & Rose, N. Amer. Fl. 23: 113. 1928. TYPE: El Salvador. Near San Salvador, 1923, *S. Calderón 1774* (holotype, NY!; isotypes, BM!, F!).

Tree to 25 m tall with bark dark grayish brown, vertically fissured, rough and scaling; twigs light brown to greenish brown, not flexuous, mostly glabrous; short shoots absent. Leaves alternate, 70–180 mm long; stipules herbaceous, light brown, narrowly linear, to 4.5 × 0.6 mm near the base, glabrous, persistent; petiole adaxially shallowly grooved, 18–46 mm long, glabrous to lightly puberulent; petiolar gland solitary, located on the middle part of the petiole, sessile, usually circular, 1.0–2.6 mm across, saucer- to cup-shaped, glabrous; rachis adaxially grooved, 40–150 mm long, glabrous to puberulent, a sessile, saucer- to doughnut-shaped gland, 0.6–1.3 mm across, between the pinnae of the upper 1 to 2 pinna pairs (Fig. 4F); pinnae (7)11 to 24 pairs per leaf, 30–70 mm long, 3–9 mm between pinna pairs; petiolules 0.8–1.5 mm long; leaflets 40 to 60 pairs per pinna, opposite, 0.5–1.2 mm distance between leaflets, linear, 3.0–5.5 × 0.6–1.1 mm, glabrous except for usually some long hairs at the base beneath, lateral veins obvious, with a midvein and 1 to 2 smaller veins from the base, base oblique, margins ciliate, apex narrowly acute to acuminate. Inflorescence a loosely flowered cylindrical spike 60–140 mm long, 1 to 3 from the leaf axis, or in terminal racemose clusters; peduncle 4–10 × 0.5–1.0 mm, glabrous to lightly puberulent; involucre absent; floral bracts linear, to 1 mm long, pubescent, early deciduous. Flowers sessile, creamy-white; calyx 5-lobed, 0.7–1.3 mm long, lightly appressed pubescent; corolla 5-lobed, 1.8–2.5 mm long, lightly appressed pubescent; stamen filaments 4.5–6.5 mm long; ovary glabrous, on a stipe to 0.3 mm long. Legumes light brown, straight, flattened, oblong, 100–160 × 16–28 mm, cartilaginous, transversely striate, glabrous, eglandular, dehiscent; stipe to 20 mm long; apex broadly acute to obtuse and usually apiculate. Seeds uniseriate, no pulp, dark reddish brown, nearly circular, strongly flattened, 6–9 mm across, smooth; pleurogram U-shaped, 1.5–3.0 mm across. Flowers: April–August, and sporadically throughout the year when moisture is available.

Distribution. Lowland forests, and moist disturbed sites below 1300 m in Guatemala, El Salvador, Honduras, Nicaragua, Costa Rica, and the states of Chiapas, Jalisco, Oaxaca, and Sinaloa, Mexico (Fig. 2).

A tall tree, sometimes entering the canopy of moist lowland forests, *Acacia centralis* is also a

common component of disturbed habitats at lower elevation through most of Central America. Most collections are from roadsides, disturbed pastures, and gallery forests. In addition to its close morphological similarity to *Acacia acatlensis* discussed above, *A. centralis* is also sympatric with five other acacia species in southern Mexico. Of these, it is easily separated from *A. compacta* and *A. mammifera,* as these species have fewer pinna pairs per leaf (fewer than 7) and fewer leaflets per pinna (fewer than 36). The erect hairs of *A. sericea,* the larger leaflets of *A. usumacintensis,* and the exfoliating bark of *A. salazari* can be used to distinguish these species from *A. centralis.*

The authors have frequently encountered specimens of *A. centralis* that were annotated *Albizia niopoides* (Benth.) Burkart. Flowering material of these two taxa is easily separated by the filament-tube and fewer stamens in *Albizia.* Fruiting material and sterile specimens also can be separated, as in *A. centralis,* the leaflets are less than 5.5 mm long and have an acuminate apex, whereas in *Albizia niopoides,* the leaflets are longer, mostly more than 5.5 mm long, and the apex is broadly acute to obtuse.

Representative specimens. COSTA RICA. **Guanacaste:** vicinity of Cañas, *Daubenmire 775* (F*); Santa Rosa National Park, *Janzen 10355* (MO*). **Puntarenas:** San Luis village, *Haber & Bello 1628* (MO*). EL SALVADOR. vicinity of Comasagua, *Allen & Armour 7257* (F, LL, NY, US*). GUATEMALA. **Petén:** Laguna Yaxja, *Harmon & Dwyer 2763* (F*, LL). **Zacapa:** Gualán, *Deam 6281* (A, F, GH*, MO, NY, UC, US, VT). HONDURAS. **Morazán:** Alrededores de la Ciudad Universitaria, *Torres 183* (NY*). **Paraiso:** 15 kms S of El Paraiso, *Molina 18420* (NY, US*). MEXICO. **Chiapas:** Siltepec, *Matuda 1584* (A, MICH*, NY, US). **Jalisco:** 5 km al SE de La Manzanilla, carretera Puerto Vallarta–Barra de Navidad, *Magallanes 906* (F, MEXU). **Oaxaca:** Pto. San Bartolo, Yautepec, *MacDougall H230* (MICH, NY*); between Limón and Zapote, *MacDougall s.n.* (NY*, US). **Sinaloa:** Mauto, Los Tepemesquites, *Ortega 5717* (GH, US). NICARAGUA. **L(on:** Volcán Momotombo, near Punta El Diablo, *Neill 480* (MO, NY). **Managua:** Las Nubes, El Crucero, *García 96* (MO*); vicinity of Managua, *Garnier 957* (F, MICH*, US); Managua, Punta Chiltepec, *Grijalva, Vanegas & Sánchez 3105* (CM, MO*); Esquipulas, *Hall & Bockus 7981* (MO, NY*, UC); Peninsula de Chiltepec, Punta Chiltepe, *Moreno 16917* (CM, MO*); along hwy. 8, km 28, *Stevens 3922* (MO*); 1.9 km from hwy. 2 on road along ridge of Sierra de Managua, *Stevens 4753* (MO*); 1 km SW of Laguna Apoyeque, *Vincelli 779* (MO*). **Masaya:** Laguna de Apoyo, *Moreno 21308* (MO). **Rivas:** Quebrada Las Cañas, near Río Escalante, *Stevens 9681* (MO).

5. Acacia compacta Rose, Contr. U.S. Natl. Herb. 8: 31. 1903. *Senegalia compacta* (Rose) Britton & Rose, N. Amer. Fl. 23: 111. 1928. TYPE: Mexico. Oaxaca: Tomellin Canyon, 24 June 1899, *J. N. Rose & W. Hough 4680* (holotype, US!; isotypes, GH!, K!, NY!).

Lysiloma standleyana Britton & Rose, N. Amer. Fl. 23: 81. 1928. TYPE: Mexico. Oaxaca: Tomellin, Sept. 1905, *J. N. Rose 10082* (holotype, NY *!, F photo!, MO photo!).

Shrub or small tree to 3(4) m tall with bark dark gray, flaking off in thin strips; twigs light brown to dark reddish brown, slightly flexuous, pubescent to glabrous, when young with minute purple glands; short shoots commonly present above the nodes, to 3 mm long, covered with acuminate stipules and old leaf bases. Leaves alternate, also commonly clustered on the short shoots, 5–30 mm long; stipules herbaceous, light brown, narrowly linear, to 3 × 0.4 mm near the base, usually glabrous, persistent; petiole adaxially grooved, 2.5–14.0 mm long, usually pubescent and with minute purple glands; petiolar gland solitary, located at or just below the lower pinna pair, sessile, circular, 0.4–1.1 mm across, doughnut-shaped, glabrous (Fig. 4H); rachis adaxially grooved, 0–20 mm long, usually pubescent and with minute purple glands, a sessile, saucer-shaped gland, 0.2–0.6 mm across, occasionally present between the upper pinna pair; pinnae 1 to 6 pairs per leaf, 6–18 mm long, 2–5 mm between pinna pairs; petiolules 0.5–1.1 mm long; leaflets 9 to 22 pairs per pinna, opposite, 0.5–0.9 mm distance between leaflets, oblong, 1.4–3.0 × 0.5–0.9 mm, glabrous above, usually lightly pubescent beneath with long hairs, lateral veins not obvious, only one vein from the base, base oblique, margins usually ciliate, apex acute to obtuse. Inflorescence a loosely flowered cylindrical spike 30–70 mm long, solitary (rarely 2 to 3) from the leaf axil; peduncle 6–13 × 0.4–0.7 mm, usually pubescent and with minute purple glands; involucre absent; floral bracts linear, to 1.5 mm long, pubescent, early deciduous. Flowers sessile, creamy-white; calyx 5-lobed, 1.1–1.7 mm long, densely appressed pubescent; corolla 5-lobed, 2–3 mm long, densely appressed pubescent; stamen filaments 5.5–7.5 mm long; ovary glabrous, on a stipe to 0.4 mm long. Legumes light yellowish brown, straight, flattened, oblong, 50–120 × 10–16 mm, cartilaginous, transversely striate, glabrous, eglandular, dehiscent; stipe to 8 mm long; apex acuminate and usually beaked. Seeds uniseriate, no pulp, purplish brown, near circular, strongly flattened, 5–8 mm across, smooth; pleurogram U-shaped, 1.3–2.2 mm across. Flowers: April–July.

Distribution. Thorn-scrub forests, thickets, rocky slopes and washes between 500 and 1600 m

elevation in the states of Puebla and Oaxaca (Fig. 3).

Acacia compacta, a much-branched shrub that rarely exceeds 3 m, has a very restricted distribution, occurring in southeastern Puebla and adjacent Oaxaca. Even there it does not appear to be a common species, relatively little material being available for study. All material examined is from xeric habitats, usually on rocky slopes and in washes, where *A. compacta* forms small thickets (Rico Arce & Rodríguez, 1998). The short shoots at many of the nodes, small leaflets (mostly less than 2.5 mm long), leaves that are less than 30 mm (mostly less than 20 mm) long, and fruits with the apex acuminate and beaked, separate *A. compacta* from other members of this group. The presence of short shoots is probably an adaptation to xeric conditions.

Acacia compacta shows variation with regard to several important characteristics. Leaf size is highly variable: the leaves that develop on the short shoots are usually smaller and have shorter petioles and fewer pinna pairs; whereas the leaves that form on fast-growing shoots are larger, the petioles sometimes reach a length of 14 mm, and 5 or 6 pinna pairs develop along the rachis. The shape and structure of the petiolar gland is also somewhat variable. The majority of the specimens have a sessile petiolar gland that is doughnut- or torus-shaped, although a few specimens have a stalked petiolar gland with a bulbous apex. Generally, these glands are found on young leaves forming on the short shoots; other leaves have sessile, doughnut-shaped glands. It is possible that *Acacia compacta* may rarely hybridize with *A. mammifera,* the only other species of this group with stalked petiolar glands.

Representative specimens. MEXICO. **Oaxaca:** 52 km al S de Tecomavaca por la carretera rumbo a la Ciudad de Oaxaca, *Salinas & Dorado F-2670* (MEXU, TEX*); 3 km al W de San Gabriel, *Salinas & Ramos F-3857* (ARIZ*, F, MEXU, RSA); 14 km al N de Cuicatlán, *Sousa, Martínez, Téllez & Magallanes 5394* (CAS*, US, WIS); a 3 km al NW de Guadalupe Los Obos, o sea a 10 km al NW de Cuicatlán, *Sousa, Téllez, Magallanes & Delgado 6912* (MICH*). **Puebla:** Alrededores del lado W del pueblo de Axusco, *Chiang, Salinas & Dorado F-2466* (ARIZ, MO, RSA*); en la desviación a San Luis Atolotitlán y Los Reyes Metzontla, *Medrano, Chiang, Davila & Villaseñor 1905* (ARIZ*, MO*); 18 km al NW de Teotitlán del Camino, a Tehuacán, *Medrano, Jaramillo, Villaseñor, Ruiz & Singer F-1172* (MO*); vicinity of San Luis Tultitlanapa, *Purpus 3193* (F, GH*, MO, NY, UC, US); 3 km al SE de San Rafael, *Rico, Ramos & Hernández 246* (CAS*, MO); Cerro de La Escalera, Ejido de San José Tilapa, *Rico, Ramos & Hernández 248* (CAS*, MO); 1 km al NE-E del limite estatal Oaxaca-Puebla por la carretera Huajuapan de León–Tehuacán, *Salinas & Dorado F-3199* (F*); a 2 km al SE de San Rafael, cerca de los limites Puebla-Oaxaca, *Sousa & Sousa 10405* (ARIZ*, TEX); a 2 km al SE de San Rafael, *Sousa, Sousa & Basurto 10430* (CAS*, TEX); Cerro Tepetroja al SW de Axusco, *Tenorio & Romero 9043* (CAS*); Jardín Botánico de Cactáceas y Suculentas de Zapotitlán de las Salinas, *Valiente-Banuet & Maeda 674* (RSA, TEX*); Jardín Botánico de Cactáceas y Suculentas de Zapotitlán de las Salinas, *Valiente-Banuet & Maeda 706* (RSA*).

6. Acacia sericea Martens & Galeotti, Bull. Acad. Roy. Sci. Bruxelles 10(2): 311. 1843. *Senegalia sericea* (Martens & Galeotti) Britton & Rose, N. Amer. Fl. 23: 111. 1928. TYPE: Mexico. Puebla: Tehuacán, alt. 6000 ft., May 1840, *H. Galeotti 3345* (holotype, BR!; isotypes, K!, P!, MICH photo!, NY photo!).

Acacia pueblensis Brandegee, Univ. California Publ. Bot. 4: 85. 1910. TYPE: Mexico. Puebla: Cerro de Solunte, alt. 7000–8000 ft., June 1909, *C. A. Purpus 3863* (holotype, UC!, MEXU photo!; isotypes, BM!, MO!, NY!, US!).

Shrub or small tree 3 to 4(6) m tall with bark dark gray, cracked and fissured, breaking away and leaving dark purplish brown, smooth areas; twigs dark brown to purplish brown, not flexuous, glabrous to lightly pubescent; short shoots absent. Leaves alternate, 30–120 mm long; stipules herbaceous, light brown, narrowly linear, to 4 × 0.4 mm near the base, glabrous to pubescent, sometimes tardily deciduous; petiole adaxially grooved, 10–30 mm long, densely pubescent with erect hairs and scattered minute purple glands; petiolar gland solitary, located along the upper half of the petiole, sessile, circular to elliptic, 0.6–1.2 mm long, saucer-shaped to cup-shaped, glabrous, sometimes absent; rachis adaxially grooved, 15–100 mm long, pubescent with erect hairs and scattered minute purple glands, a sessile, saucer-shaped gland, 0.5–1.1 mm across, between the pinnae of the upper 1 or 2 pinna pairs; pinnae 5 to 13 pairs per leaf, 25–49 mm long, 4–8 mm between pinna pairs; petiolules 1.5–2.1 mm long; leaflets 14 to 35(44) pairs per pinna, opposite, 0.7–1.3 mm interval between leaflets, linear, 2.5–5.0 × 0.8–1.5 mm, pubescent on both surfaces with appressed to erect hairs, lateral veins obvious with only one vein from the base, base oblique, margins ciliate, apex acute to obtuse. Inflorescence a densely flowered cylindrical spike 30–80 mm long, solitary from the leaf axil; peduncle 3–20 × 0.7–1.2 mm, densely pubescent with erect hairs; involucre absent; floral bracts linear, to 3.5 mm long, densely pubescent, deciduous. Flowers sessile, creamy white; calyx 5-lobed, 1.5–2.2 mm long, densely pubescent with erect hairs; corolla 5-lobed, 2.0–3.0 mm long, densely pubescent with erect hairs; stamen filaments 6–8 mm long; ovary glabrous, on a stipe to 0.3 mm long. Legumes

light yellowish brown, straight, flattened, oblong, 90–170 × 15–24 mm, cartilaginous, transversely striate, pubescent, eglandular but usually with minute purple glands when young, dehiscent; stipe to 14 mm long; apex acuminate and apiculate to 5 mm long. Seeds uniseriate, no pulp, dark purplish brown, nearly circular, strongly flattened, 5.0–8.5 mm across, smooth; pleurogram U-shaped, 1.5–2.5 mm across. Flowers: February–June.

Distribution. Rocky desert and dry thorn-scrub forests from 1100 to 2000 m elevation in Puebla and Oaxaca, Mexico (Fig. 2).

A small tree, mostly 3 or 4 m tall, *Acacia sericea* is known from southeastern Puebla and adjacent Oaxaca. It occurs at higher elevation, usually above 1100 m elevation, in desert and thorn-scrub forests. Most collections are from roadsides, usually in dry, disturbed habitats, and many are from the Tehuacán valley (Rico Arce & Rodríguez, 1998). Based on the number of specimens available for study, this taxon is not a common component of the vegetation.

Acacia sericea is distinct from most other members of this group. The dense, erect pubescence on most parts of the plant makes it easy to distinguish this taxon. The leaf rachis, the pinna rachis, and usually the petiole are densely pubescent with erect hairs that exceed 0.3 mm in length. The leaflets are mostly pubescent with erect to slightly appressed pubescence, whereas the mature fruits are short pubescent. Also, the calyx and corolla are pubescent with erect hairs, as are the floral bracts, which are commonly longer than those found in other members of this group.

Acacia sericea possibly hybridizes with *A. acatlensis* in areas where they are sympatric. Occasional specimens were encountered with reduced pubescence and many leaflet pairs per pinna, characteristics usually associated with *A. acatlensis.*

Representative specimens. MEXICO. **Oaxaca:** Cuesta inferior de Salome, Districto de Cuicatlán, *Conzatti 5321* (NY*); Cuesta de ruta Vieja, *Smith 430* (GH*); a 8 km al NE de Teotitlán del Camino, carretera a Huautla, *Sousa 6923* (CAS, MEXU, MO, UC*); a 12 km al N de Tonaltepec, *Sousa & Magallanes 8917* (ISC*); a 8 km al NE de Teotitlán del Camino, en el camino a Huautla, *Sousa, Martínez, Téllez & Magallanes 5396* (CAS, MEXU*, US, WIS); a 9 km al NE de Teotitlán del Camino, *Sousa, Martínez, Téllez & Magallanes 5402* (CAS*, US); a 9 km al N de la desviación a Tonaltepec, *Sousa, Ramos & Téllez 6131* (CAS, MO, SD*, WIS); a 8 km al E-NE de Teotitlán del Camino, camino a Huautla, *Sousa, Téllez, Germán & Rico 8083* (CAS*); Cañada de Carrizalillo, Cerro Verde, *Tenorio, Romero, Martínez & Tenorio 6983* (F, MEXU*); 7.6 km al E de Teotitlán, carretera a Huautla de Jiménez, *Torres & Martínez 6499* (MEXU*, MO). **Puebla:** Cerros calizos al NE de Tehuacán, *Chiang, Villaseñor & Durán F-2024* (MO, NY*); Tehuacán, *Conzatti 2176* (F*, US); 3 mi. N of the city limits of Tehuacán, *Hansen, Hansen & Nee 1728* (LL, MICH, US*, WIS); about 10 mi. N of Tehuacán on main road toward Orizaba, *Hughes, Lewis & Contreras 1320* (MEXU, NY*); Cerro de Solumta, *Purpus 3863* (MO*); Tehuacán, *Purpus 5845* (F, MO, NY*, UC, US); Tehuacán, *Purpus 10682* (NY, US*); Acatepec, SW Zapotitlán, *Sousa 2668* (CAS*, US); Ladera W de Cerro Grande Mpio. Caltepec, *Tenorio & Romero 5424* (CAS*); Barranca de Los Membrillos, al SW de Caltepec, *Tenorio, Torres & Romero 3784* (CAS*).

7. **Acacia mammifera** Schlechtendal, Linnaea 12: 563. 1838. *Senegalia mammifera* (Schlechtendal) Britton & Rose, N. Amer. Fl. 23: 112. 1928. TYPE: Mexico. Hidalgo: Barranca de Acholoya, *C. Ehrenberg s.n.* (holotype, HAL!; isotype, UC!).

Shrub or small tree to 5 m tall with bark dark gray, shallowly fissured; twigs light brown to purplish brown, not flexuous, usually puberulent and, when young, commonly with minute purple glands; short shoots absent. Leaves alternate, 30–130 mm long; stipules herbaceous, light brown, narrowly triangular, 2.5 × 0.6 mm near the base, glabrous to puberulent, persistent; petiole adaxially grooved, 8–50(70) mm long, usually lightly puberulent and commonly with minute purple glands; petiolar gland solitary, located between the lower pinna pair or rarely along the upper half of the petiole, stalked, circular, 0.4–0.8 mm across, apex globose, glabrous (Fig. 4D); rachis adaxially grooved, 10–70 mm long, puberulent and commonly with minute purple glands, with a stalked gland with a globose apex, 0.4–0.6 mm across, between most pinna pairs; pinnae 1 to 6(9) pairs per leaf, 30–85 mm long, 6–15(25) mm between pinna pairs; petiolules 2.0–2.8(4.5) mm long; leaflets 10 to 26(33) pairs per pinna, opposite, 1–5 mm between leaflets, oblong, 4–12 × 1.5–3.5(4.5) mm, loosely pubescent on both surfaces with appressed hairs, commonly purplish above, light green to purplish green beneath, lateral veins obvious with a midvein and 1 to 3 smaller veins from the base, base oblique, margins ciliate, apex obtuse to broadly acute. Inflorescence a loosely flowered cylindrical spike 30–90 mm long, solitary (rarely 2) from the leaf axil; peduncle 6–15 × 0.7–1.1 mm, puberulent and with minute purple glands; involucre absent; floral bracts linear, to 1.4 mm long, pubescent, early deciduous. Flowers sessile, creamy-white; calyx 5-lobed, 1.3–2.0 mm long, lightly appressed pubescent; corolla 5-lobed, 2.2–3.5 mm long, lightly appressed pubescent; stamen filaments 6.5–8.5 mm long; ovary glabrous, on a stipe to 0.3 mm long. Legumes light yellowish brown to dark greenish

brown, straight, flattened, oblong, 80–240 × 18–35 mm, cartilaginous, transversely striate, glabrous, eglandular, dehiscent; stipe to 12 mm long; apex acuminate and usually beaked. Seeds uniseriate, no pulp, dark brown, nearly circular, strongly flattened, 8.0–10.5 mm across, smooth; pleurogram U-shaped, 3–4 mm across. Flowers: April–June.

Distribution. Thorn-scrub forests and from the pinyon-juniper zone in dry thickets, and rocky slopes from 1300 to 2700 m elevation in eastern Mexico from the states of Tamaulipas and Nuevo León south to Oaxaca (Fig. 3).

Acacia mammifera is widely distributed throughout the central part of Mexico from the state of Oaxaca, north to Tamaulipas and Nuevo León. It does not appear to be a common species, however, many of the collections being from near the same localities in the various states. All collections examined are from above 1300 m, many from near the pinyon-juniper zone, or from rocky slopes and dry thickets, mostly associated with thorn-scrub vegetation. The few pinna pairs, the large leaflets with appressed hairs on both surfaces, the dull purple color on the upper surface of the leaflets, the narrowly triangular stipules, and the stalked petiolar glands can be used to separate *Acacia mammifera* from other members of this group.

This wide-ranging species is not extremely variable morphologically, but one unusual specimen from Puebla, Mexico, was encountered [*E. M. Martínez S. 21665* (MEXU)]. This was a small, compact plant with the small leaves and leaflets of *A. compacta,* but was similar to *A. mammifera* in lacking short shoots, having narrowly triangular stipules, a stalked petiolar gland with a bulbous apex, and leaflets that were purplish and pubescent on both surfaces. Occurring at an elevation of 2300 m, this specimen was probably outside the natural range of *A. compacta.* The status of this possible hybrid must await additional field studies.

Representative specimens. MEXICO. **Guanajuato:** Rancho Beltrán, 10 km al S de Xichú, *Ventura & López 6778* (CAS, MEXU*). **Hidalgo:** Barranca de Tuzanapa, Zacualtipán, *González 824* (MEXU*); Barrancas de Tolantongo, Cardonal, *Hernández 3775* (MO*); 6 km al N de Zoquital, *Hernández, Cortés & Hernández 6036* (CAS, MO*); a 3 km al N de Molanguito, Tolantongo, Cardonal, *Medrano, Hiriart & Ortíz 10083* (MEXU*, MO); Sierra de la Mesa, *Rose, Painter & Rose 9126* (GH, NY, US*). **Nuevo León:** a 24 km al S de Cd. Linares, *Castillón 687* (MO, NY*); Cañon la Boca, camino a Cola de Caballo, Laguna de Sánchez, *Villarreal, Carranza & Moreno 2736* (TEX*). **Oaxaca:** a 3 km sobre el camino Tamazulapán–Chilapa, *Mendoza 930* (CAS, MO, MU*, WIS); a 4 km al SW de Tamazulapán, sobre la carretera Tamazulapán–Chilapa de Díaz, *Rico, Torres & Cedillo 333* (CAS*, MO). **Puebla:** Barranca Honda al NW de Caltepec, *Tenorio & Romero 5777* (ARIZ*, CAS, CM, TEX, WIS); Cerro Tepearco, al E de el Rancho de Tlacuiloltepec, *Tenorio & Romero 8814* (MO*, RSA, TEX). **Querétaro:** about 80 km NE of Querétaro on road to Pinal de Amoles, *McVaugh 10364* (GH, LL, MICH*, MO, TEX, US); 3–4 km al Poniente de La Parada, *Servin 117* (CAS*, MEXU); Cuesta Colorada, km 21 de la carretera Vizarrón–Jalpán, *Tenorio & Hernández 272* (CAS, ILL*, MO). **San Luis Potosí.** 5 km W of jct. of hwy. 86 with roads to Rayón and Cárdenas, 82 km W of Valles, *Roe & Roe 2220* (NY*, WIS). **Tamaulipas:** *ca.* 5 km N of La Joya de Salas, trail to Carabanchel, *Martin 129* (MICH*); 8 mi. E of Dulces Nombres, *Meyer & Rogers 2640* (GH, MO*); 15 km al N de Tula, *Puig 4741* (MEXU*).

8. Acacia durangensis (Britton & Rose) Jawad, Seigler & Ebinger, comb. nov. Basionym: *Senegalia durangensis* Britton & Rose, N. Amer. Fl. 23: 112. 1928. TYPE: Mexico. Durango: San Ramón, 21 Apr.–18 May 1906, *E. Palmer 107* (holotype, NY*!, MEXU photo!; isotypes, F!, MO!, UC!, US!).

Shrub or small tree to 5 m tall with bark of main trunk dark gray, shallowly fissured; twigs light brown, not flexuous, puberulent; short shoots absent. Leaves alternate, 65–160 mm long; stipules herbaceous, light brown, narrowly triangular, to 2.5 × 0.7 mm near the base, puberulent, persistent; petiole adaxially grooved, 30–50 mm long, puberulent and with erect hairs to 0.2 mm long; petiolar gland solitary, located near the middle of the petiole, sessile, elliptical, 1.1–2.2 mm across, apex irregularly raised, glabrous (Fig. 4A); rachis adaxially grooved, 50–130 mm long, puberulent and usually with minute purple glands, a sessile, flattened gland, 0.4–0.8 mm across, between the pinnae of the upper 1 to 2 pinna pairs; pinnae 6 to 13 pairs per leaf, 60–85 mm long, 8–14 mm between pinna pairs; petiolules 2.5–4.0 mm long; leaflets 28 to 36 pairs per pinna, opposite, 1.3–2.1 mm between leaflets, linear, 5.0–7.5 × 1.3–2.1 mm, loosely pubescent on both surfaces with appressed hairs, commonly purplish above, light green to purplish green beneath, lateral veins obvious with a midvein and 1 to 2 smaller veins from the base, base oblique, margins ciliate, apex obtuse to acute. Inflorescence a loosely flowered cylindrical spike 60–120 mm long, solitary (rarely 2) from the leaf axil, or rarely in short racemose clusters; peduncle 5–15 × 1.0–1.8 mm, puberulent; involucre absent; floral bracts linear, to 1 mm long, pubescent, early deciduous. Flowers sessile, creamy-white; calyx 5-lobed, 1.0–1.4 mm long, densely appressed pubescent; corolla 5-lobed, 2.0–2.5 mm long, densely appressed pubescent; stamen filaments 5.5–7.5 mm long; ovary glabrous, on a stipe to 0.4 mm long. Legumes dark reddish brown, straight, flattened,

oblong, 80–120 × 16–22 mm, cartilaginous, transversely striate, glabrous to lightly puberulent, eglandular, dehiscent; stipe to 10 mm long; apex acuminate and usually beaked. Seeds not seen. Flowers: April–June.

Distribution. Thorn-scrub forests and dry thickets, 1600 to 2200 m elevation in the states of Durango and Chihuahua, Mexico (Fig. 3).

Few specimens of *Acacia durangensis* are available for study; we saw the type and two others. This is the only species of this group known from Durango, and it occurs in a region that has been poorly collected. Superficially, *A. durangensis* is similar to *A. mammifera,* the leaflets being purplish above, light green beneath, with obvious veins and loosely pubescent on both surfaces with appressed hairs. Leaves with 6 to 13 pinna pairs, pinnae with 28 to 36 leaflets, and the sessile petiolar gland that is elliptical in outline and with a raised apex separate this taxon from *A. mammifera.* The petiolar gland is the most distinctive feature of *A. durangensis.* Other species of this group have a flat or doughnut-shaped gland, or the gland is stalked. In *A. durangensis,* in contrast, the sessile gland appears as an elliptical mound, with a few indentations. On herbarium specimens, this gland has a purple color, and rarely a few long hairs on its surface.

Though none were observed on the few specimens available, it is possible that plants of *Acacia durangensis* occasionally may have prickles. If present, this would suggest that *A. durangensis* is more closely related to *A. macilenta* and other members of the *Acacia* series *Vulgares* that commonly have prickles. Also, the petiolar gland of *A. durangensis* is similar to those found in many members of *Acacia* series *Vulgares.*

Representative specimens. MEXICO. **Chihuahua:** between El Tejebán and Río Unique, *Bye, Davis, Randolph & Gerson 12774* (MEXU*). **Durango:** El Pino 20 km de el entronque a Sapioris, con la Brecha Coyotes–San Miguel de Cruces, Mpio. Tayoltita, 24°31′N, 105°49′W, *Tenorio, Romero & Ramamoorthy 6323* (TEX*).

9. Acacia willardiana Rose, in Vasey & Rose, Contr. U.S. Natl. Herb. 1: 88. 1890. TYPE: Mexico. Sonora: rocky islands and ledges on the coast of Guaymas, 1–2 Apr. 1890, *E. Palmer 164* (holotype, US!).

Prosopis heterophylla Bentham, London J. Bot. 5: 82. 1846. *Senegalia heterophylla* (Bentham) Britton & Rose, N. Amer. Fl. 23: 114. 1928. TYPE: Mexico. Sonora: Alta, 1830, *T. Coulter s.n.* (holotype, TCD) [not *Acacia heterophylla* Willdenow, 1806].

Tree to 10 m tall with bark smooth, white to reddish yellow, exfoliating and papery; twigs light gray, becoming dark reddish purple, not flexuous, glabrous; short shoots absent. Leaves alternate, 30–400 mm long; stipules herbaceous, light brown, narrowly linear, 1.1 × 0.2 mm near the base, glabrous, tardily deciduous; petiole flattened, not grooved, 20–400 mm long, usually glabrous; petiolar gland solitary, located between to just below the lower pinna pair, sessile, circular, 0.3–0.7 mm across, doughnut-shaped, glabrous (Fig. 4G); rachis flattened, not grooved, 0–100 mm long, glabrous, glands absent; pinnae 1 (rarely 3) pair per leaf, 16–80 mm long; petiolules 2.5–8 mm long; leaflets 4 to 20 pairs per pinna, opposite, 1–5 mm distance between leaflets, oblong to elliptic, 3.0–7.5(12.0) × 1.0–2.3 mm, glabrous to rarely lightly pubescent with appressed hairs on both surfaces, lateral veins not obvious with only one vein from the base, base oblique, margins lightly ciliate, apex narrowly acute to acuminate. Inflorescence a loosely flowered cylindrical spike 30–90 mm long, solitary from the leaf axil, or in short racemose clusters. Peduncle 5–25 × 0.4–0.8 mm, glabrous or nearly so; involucre absent; floral bracts linear, to 1 mm long, glabrous to lightly pubescent, early deciduous. Flowers sessile, creamy-white; calyx 5-lobed, 1.3–2.2 mm long, glabrous; corolla 5-lobed, 2.4–3.6 mm long, glabrous; stamen filaments 6–8 mm long; ovary glabrous, on a stipe to 1 mm long. Legumes light yellowish brown, straight, flattened, oblong, 70–180 × 8–22 mm, chartaceous, irregularly striate, glabrous, eglandular, dehiscent; stipe to 14 mm long; apex obtuse. Seeds uniseriate, no pulp, dark brown, nearly circular, strongly flattened, 6–11 mm across, smooth; pleurogram usually absent, when present, U-shaped, about 2 mm across. Flowers: February–June.

Distribution. Arid hills, rocky slopes and washes in desert scrub vegetation between sea level and 500 m elevation in Sonora, Mexico (Fig. 3).

A common species at lower elevations in the state of Sonora, *Acacia willardiana* is a very obvious component of the desert scrub of this region because of it nearly white, to yellowish, to almost reddish, papery, exfoliating bark. Being so obvious, this species is commonly collected; numerous specimens are from the vicinity of Guaymas. The majority of the specimens lack pinnae, which are early deciduous; only the elongated, flattened petioles persist. Although flowering is common from February through May, it may occur at other times if moisture is available.

Acacia willardiana is easily separated from other members of this group. The most obvious differ-

ences are the elongated, flattened petioles that may reach 400 mm in length, and the leaves with usually only one pair of pinnae, though rarely two or three may be present. This species has very small, tardily deciduous stipules (1.1 mm long or less), and the fruit valves are papery with more irregular striations than those found in other members of this group. The characteristic papery, exfoliating bark is shared only with *A. salazari*, a species of this group restricted to central Mexico.

Bentham (1846) tentatively assigned this taxon to *Prosopis heterophylla* based on a single fruiting specimen. He suggested that the general habit of the plant was more like that of *Prosopis* than any other genus, and mentioned the almost phyllodinous vertical expansion of the petiole. Based on flowering material, Vasey and Rose (1890) realized that this taxon was an *Acacia* and used the name *A. willardiana*, as Bentham's name was preoccupied.

Vassal (1972) noted that *A. willardiana* is a species with authentic phyllodes and suggested that, if he had seen flowering material, Bentham would have placed this species in the "Juliflorae." Vassal considered this species to be a member of his section *Heterophyllum*, subsection *Spiciferae*, and to have affinities to this predominantly Australian group. In our view, homology between the phyllodes of that group and the apparent petioles of *A. willardiana* should be examined more thoroughly. Further, in most other characteristics, this species differs little from other members of the *A. coulteri* group discussed in the present study.

Representative specimens. MEXICO. **Sonora:** rocky hill N of Guaymas, *Blakley B-820* (ASU*); ½ mi. from top of microwave tower hill at Guaymas, *Brunner 57* (ARIZ*); Bahía de San Carlos, ca. 24 km NW of Guaymas, *Carter & Kellogg 3246* (SD*); ca. ½ mi. SW of Hotel Playa de Cortés, Miramar, *Felger 5513* (ARIZ*); Isla Tiburón, *Felger 9151B* (ARIZ*, SD); Sierra Seri, Hast Eemla, *Felger, Drees & Moser 74-8* (ARIZ*); Bahía Colorado, arroyo at E base of Morro Colorado, *Felger & Hamilton 15638* (RSA*); Ensenada Grande, San Pedro Bay, *Felger, Russell & Kleine 11588* (ARIZ, SD*); Sierra Bojihuacame SE of Cd. Obregón, *Gentry 14483* (ARIZ*, US); 16 mi. S of Hermosillo, *Gentry & Engard 551A* (ASU*); 5 mi. N of Guaymas near hwy. 15, *Henrickson 1565* (RSA*); Bahía San Carlos, *Howard & Sphon s.n.* (RSA*); Hermosillo, *Jones 22495* (MO, POM*); microwave tower, 60 mi. N of Navijoa, *Joseph s.n.* (ARIZ*); hillside N of Guaymas, *Knobloch 2325* (SD*); Guaymas, San Carlos Bay, road to Catch 22 beach, *Landrum & Landrum 6315* (ASU*, MO, RSA); limestone quarry SE of Hermosillo, *Martin s.n.* (ARIZ*); Ardilla Island, Guaymas Harbor, *Moran 4017* (SD*, US, WIS); rte. 15, ca. 25 mi. S of Hermosillo, *Pinkava P12788* (ARIZ, ASU*); dry hillside slopes near Torres, *Whitehead M250* (ARIZ*).

10. Acacia millefolia S. Watson, Proc. Amer. Acad. Arts 21: 427. 1886. *Senegalia millefolia* (S. Watson) Britton & Rose, N. Amer. Fl. 23: 111. 1928. TYPE: Mexico. Chihuahua: Hacienda San José, near Batopilas, Aug. 1885, *E. Palmer 45* (lectotype, designated by Isely (1969), GH!; isolectotypes, MEXU!, NY!).

Shrub or small tree to 3 m tall with bark gray, smooth when young, becoming fissured into square plates 1–2 cm across; twigs light brown to greenish brown, not flexuous, usually lightly puberulent; short shoots absent. Leaves alternate, 60–230 mm long; stipules herbaceous, light brown, narrowly linear, 6.5 × 0.5 mm near the base, usually glabrous, persistent; petiole adaxially grooved, 30–75 mm long, usually glabrous; petiolar gland absent; rachis adaxially grooved, 50–190 mm long, glabrous to lightly pubescent; a stalked gland with a globose apex, 0.3–0.9 mm across, between the pinnae of the upper 1 to 2 pinna pairs; pinnae (2)6 to 14 pairs per leaf, 30–55 mm long, 10–28 mm between pinna pairs; petiolules 2.0–4.0 mm long; leaflets 20 to 35(37) pairs per pinna, opposite, 0.8–1.6 mm between leaflets, oblong, 2.0–6.5 × 0.7–1.4 mm, glabrous above, lightly pubescent beneath with appressed hairs, lateral veins not obvious with only one vein from the base, base oblique, margins sometimes ciliate, apex acuminate. Inflorescence a loosely flowered cylindrical spike 30–75 mm long, solitary (rarely 2 to 3), from the leaf axil; peduncle 5–15 × 0.3–0.8 mm, glabrous to lightly puberulent; involucre absent; floral bracts linear, to 1.3 mm long, glabrous to lightly pubescent, early deciduous. Flowers sessile, creamy-white; calyx 5-lobed, 1.1–1.6 mm long, glabrous; corolla 5-lobed, 2.0–2.7 mm long, glabrous; stamen filaments 4.5–6.5 mm long; ovary glabrous, on a stipe to 0.4 mm long. Legumes light yellowish brown, straight, flattened, oblong, 70–170 × 12–21 mm, chartaceous, irregularly striate, glabrous, eglandular, dehiscent; stipe to 12 mm, apex acute to obtuse. Seeds uniseriate, no pulp, dark brown, nearly circular, strongly flattened, 6.2–9.5 mm across, smooth; pleurogram U-shaped, 2–3 mm across. Flowers: June–August.

Distribution. Desert grasslands, rocky slopes, subtropical scrub, and open oak woodlands from 700 to 1700 m elevation in southern Arizona, south through Sonora to western Chihuahua, Mexico (Fig. 3).

A shrub or small tree, mostly less than 3 m tall, *Acacia millefolia* is relatively common in desert grassland and desert scrub vegetation in extreme southern Arizona and adjacent Sonora, Mexico. Commonly collected along the steep sides and

floors of canyons, *A. millefolia* is rarely a dominant member of the vegetation, most collections indicating scattered individuals.

Though numerous specimens are available from throughout most of the geographic range of this taxon, no specimens, other than the type collection, are known from Chihuahua. This taxon may be extremely rare in southwestern Chihuahua, or it is possible that the collecting data on the type specimens are incorrect. This collection is more than 100 km east of any specimens of *A. millefolia* seen by the present authors.

Acacia millefolia is distinct from other members of this group, as it lacks a petiolar gland and possesses a stalked rachis gland between the upper pairs of pinnae. The only other taxa of this group within the range of *A. millefolia* are *A. willardiana* and *A. russelliana*. *Acacia millefolia* is easily distinguished from *A. willardiana*, which has papery, exfoliating bark and leaves with only 1 to 3 pinna pairs. Leaflets with appressed hairs, the stalked rachis glands, and the persistent stipules of *A. millefolia* separate it from *A. russelliana*. In addition, *A. millefolia* is normally a shrub found above 700 m elevation, whereas the other species are usually trees and commonly occur at lower elevations.

Representative specimens. MEXICO. **Sonora:** 3 km by road S of Nacozari, *Felger 3596* (ARIZ*, MICH, SD); 4 mi. N of Colonia Oaxaca, *Hastings & Turner 65-25* (ARIZ, SD*); 5 mi. E of Mina Verde, *Shreve 6753* (F*, MICH, MO); Río Bavispe, Colonia Oaxaca, *White 679* (ARIZ*, GH, MICH); Cañon de Bavispe, *White 3019* (ARIZ, GH, MICH*); 9 mi. W of La Angostura, *White 4034* (ARIZ, GH, MICH*); Puerto del Molino Quemado, E of Colonia Morelos, *White 4511* (ARIZ*, GH, MICH); 3 mi. S of Divisaderos, *Wiggins 7457* (A, ARIZ, CH, F*, UC, US). U.S.A. **Arizona:** Cochise Co.: foothills of the Peloncillo Mts., T22S R32E S32, *Kluever s.n.* (ARIZ*). Pima Co.: Box Canyon, Santa Rita Mts., *Goodding 89-53* (ARIZ*); Chimney Creek, Rincón Mts., *Kearney & Peebles 10461* (ARIZ*, MICH, UC, US); Box Canyon, N end of Santa Rita Mts., *McKeighen s.n.* (ARIZ, ASU*, UC); 7 mi. W of hwy. 83 on Greaterville–Madera Canyon Road, *McLaughlin 64* (ARIZ*); vic. of Helvetia, Santa Rita Mts., *Mittleman 373* (ARIZ, ASU*); Greaterville Road, 5.4 mi. W of hwy. 83, *Pinkava, Keil & Lehto 14487* (ASU*); foothills of the Santa Rita Mts., *Pringle s.n.* (A, F, GH, MICH, NY*, US); Total Wreck Mines, *Thornber 107* (MO, POM, UC*, US); Ophir Gulch, 4 mi. N and 2 mi. W of Sonoita, *Tramontano T-25* (ARIZ*); Saguaro National Monument, *Turner & Gunzel 78-111* (ARIZ*, SD). Santa Cruz Co.: about ¾ mi. N of Sonoita Creek above Rio Rico, *Kaiser 1394* (ARIZ*). **New Mexico:** Hidalgo Co.: about 0.5 mi. E of Arizona, about 50 m N of Mexico border, wedge Sec. 24 T24S, R22W, *Spellenberg & Repass 5371* (NMC).

11. Acacia salazari (Britton & Rose) Lundell, Contr. Univ. Michigan Herb. 4: 8. 1940. *Senegalia salazari* Britton & Rose, N. Amer. Fl. 23: 113. 1928. TYPE: Mexico. Michoacán: Xochiapa, 13 Apr. 1912, *F. Salazar s.n.* (holotype, US!; isotypes, MEXU!, NY!).

Tree to 15 m tall with bark yellow to red or light gray, exfoliating and papery; twigs greenish brown to light reddish brown, not flexuous, usually glabrous; short shoots absent. Leaves alternate, 50–180 mm long; stipules herbaceous, light brown, narrowly linear, 4.5 × 0.6 mm near the base, glabrous, persistent; petiole adaxially grooved, 20–50(60) mm long, mostly glabrous; petiolar gland solitary, located between the lower pinna pair, sessile, nearly circular, 0.5–1.3 mm across, globose to doughnut-shaped, absent on most leaves; rachis adaxially grooved, 35–140 mm long, glabrous to lightly puberulent, a sessile, doughnut- to saucer-shaped gland, 0.5–1.0 mm across, between the pinnae of the upper 1 or 2(3) pinna pairs; pinnae 7 to 18 pairs per leaf, 35–63 mm long, 4–12 mm between pinna pairs; petiolules 2.4–4.0 mm long; leaflets 20 to 38 per pinna, opposite, 0.8–1.5 mm between leaflets, linear, 2.5–6.4 × 0.9–1.3 mm, usually lightly pubescent with appressed hairs beneath, commonly light greenish purple and glabrous above, lateral veins usually not obvious with only 1(2) vein(s) from the base, base oblique, margins usually ciliate, apex narrowly acute to acuminate. Inflorescence a loosely flowered, cylindrical spike 45–110 mm long, 1 to 3 from the leaf axil, or rarely in terminal racemose clusters; peduncle 5–10 × 0.5–1.0 mm, lightly puberulent; involucre absent; floral bracts linear, to 1 mm long, puberulent, early deciduous. Flowers sessile to rarely short stipitate, creamy-white; calyx 5-lobed, 1.5–2.3 mm long, lightly appressed pubescent; corolla 5-lobed, 2.5–3.5 mm long, lightly appressed pubescent; stamen filaments 4.5–7.0 mm long; ovary glabrous, on a stipe to 0.3 mm long. Legumes yellowish brown, straight, flattened, oblong, 115–180 × 21–35 mm, cartilaginous, transversely striate, glabrous, eglandular, dehiscent; stipe to 13 mm long; apex obtuse. Seeds uniseriate, no pulp, dark reddish brown, circular to oblong, strongly flattened, 10.0–14.5 × 7–12 mm, smooth; pleurogram U-shaped, 1.2–3.0 mm across. Flowers: April–June.

Distribution. Thorn-scrub thickets, and disturbed dry forests from near sea level to 1800 m (but mostly above 1000 m) elevation in the states of Guererro, México, Michoacán, Morelos, Oaxaca, and Puebla, Mexico (Fig. 3).

A small tree, not exceeding 15 m in height, *Acacia salazari* is restricted to dry habitats, particularly thorn-scrub forests of southern Mexico. Many collections are from above 1000 m elevation, but a

few individuals are reported from near sea level. *Acacia salazari* is similar to *A. acatlensis* with which it is sympatric nearly throughout its entire range. Both have leaves with many pinna pairs, and leaflets of similar size that are lightly pubescent with appressed hairs beneath and commonly light greenish purple and glabrous above. *Acacia acatlensis*, however, has minute purple glands along the petiole, rachis, and at the base of most leaflets; pinnae with more than 36 pairs of leaflets; petiolules that are mostly less than 2.0 mm long; and dark gray, fissured bark. *Acacia salazari*, in contrast, lacks the small purple gland and usually has fewer than 38 pairs of leaflets per pinna, petiolules that usually exceed 2.5 mm in length, and yellow to reddish bark that is papery and exfoliating. Some specimens with a mixture of characteristics of these two species suggest that they probably hybridize in Guerrero. Determination must await field studies.

Although *A. salazari* is similar to other species of this group from southern Mexico, particularly *A. centralis* and *A. usumacintensis*, the papery, exfoliating bark allows for easy separation. Also, both of these species have pinnae with more than (36)40 leaflet pairs, whereas *A. salazari* has 38 or fewer leaflet pairs per pinna. *Acacia usumacintensis* has large, flattened petiolar glands that are located on the lower half of the petiole, while *Acacia salazari* commonly lacks petiolar glands. Occasionally, petiolar glands are found on a few leaves of some specimens; these are globose to doughnut-shaped, and located between the lower pair of pinnae. These glands are not common; most specimens lack petiolar glands altogether. On the remaining specimens, only one or two of the leaves have petiolar glands, and many are globose, not doughnut-shaped as is typical in most taxa of this group. *Acacia salazari* and *A. millefolia* are the only taxa in this group that usually lack petiolar glands.

Representative specimens. MEXICO. **Guerrero:** creek banks N of Chilpancingo, *Clark 7235* (MO*); 21 km al NE de Chilpancingo, *Delgado & García 1085* (CAS*, F, MEXU, MO, NY, WIS); a 20 km al N de Chilpancingo, camino a Iguala, *Martínez 557* (F*); N de Zumpango del Río, *Rico & Funk 204* (CAS*); 24 km al N de Zumpango del Río hacia Iguala, *Torres, Tenorio & Romero 1243* (CAS*, MO). **México:** El Jumate, a 1 km al S después del cruzero San Antonio del Rosario, *Rico & Martínez 848* (NY*). **Michoacán:** between Río Tepalcatepec and Arteaga, *McVaugh 22520* (CAS, MICH*, MO, NY); en Las Cañas, cerca de la desviación al Infiernillo, carretera Nueva Italia–Playa Azul, *Soto 1081* (CAS, MO*); carretera Nueva Italia–Arteaga, *Soto & Aureolea 7745* (MEXU*). **Morelos:** yard, Apuyeca, *Clausen 6036* (CU*). **Oaxaca:** Rancho El Mezquite, a 7 km al S de Teotitlán, *Sousa 6934* (CAS, MO, SD, UC*); Teotitlán del Camino, en la orilla del Pueblo, *Sousa, Téllez, Germán & Rico 8060* (CAS, MO*); a 3 km al E-NE de Teotitlán del Camino, carretera a Huautla, *Sousa, Téllez, Germán & Rico 8079* (CAS*, TEX). **Puebla:** 20 mi. SE of Tehuacán on road to Teotitlán del Camino, *Anderson & Anderson 5332* (MICH*); 3.5 km al N de Teotitlán, por el camino rumbo a Vigastepec, *Cornelo 25* (MO*); Piaxtla, *Huerta s.n.* (NY*); just N of Coxcatlán along hwy. 131, *Lavin, Sundberg, Hardison & Whittemore 4610* (TEX*); El Papayo, a 16 km al NW de Amatitlán, *Sousa, Pérez & Ruiz 4339* (NY*); Ahuehuetilla, a 20 km al NW de Acatlán, *Sousa, Téllez & Magallanes 5418* (MICH*, MO); hwy. 190, 17 mi. from the Oaxaca border in Puebla, *Trott, Case, Thurm, Dunn, Hess & Dziekanowski 140* (MO, NY*).

12. Acacia coulteri Bentham, in A. Gray, Pl. Wright. 1: 66. 1852. *Senegalia coulteri* (Bentham) Britton & Rose, N. Amer. Fl. 23: 112. 1928. TYPE: Mexico. Hidalgo: Zimapán, *T. Coulter s.n.* (holotype, K!, F photo!, GH photo!, MEXU photo!, MICH photo!, MO photo!, NY photo!, US photo and fragment!).

Shrub or small tree to 15 m tall with bark dark gray, shallowly furrowed; twigs light brown to greenish brown, not flexuous, glabrous to lightly appressed puberulent; short shoots absent. Leaves alternate, 50–150 mm long; stipules herbaceous, light brown, narrowly linear, 2.1 × 0.4 mm near the base, glabrous, tardily deciduous; petioles adaxially shallowly grooved, 25–55 mm long, usually lightly appressed puberulent; petiolar gland solitary, located on the upper third of the petiole and commonly just below the first pinna pair, sessile, nearly circular, 0.5–1.6 mm across, doughnut-shaped, glabrous, rarely absent (Fig. 4E); rachis shallowly grooved adaxially, 20–100 mm long, lightly puberulent, a sessile, cup-shaped gland, 0.4–0.9 mm across, between upper pinna pair; pinnae 5 to 11 pairs per leaf, 40–90 mm long, 6–12 mm distance between pinna pairs; petiolules 3–5 mm long; leaflets 18 to 35 pairs per pinna, opposite, 1.5–2.3 mm interval between leaflets, oblong, 4.5–7.5 × 1.4–2.1 mm, glabrous above, lightly appressed pubescent beneath, lateral veins obvious with a midvein and occasionally one other vein from the base, base oblique, margins ciliate, apex broadly acute to obtuse. Inflorescence a loosely flowered cylindrical spike 50–90 mm long, 1 to 4 from the leaf axil, or rarely in terminal racemose clusters; peduncle 7–13 × 0.5–1.0 mm, usually puberulent; involucre absent; floral bracts linear, to 1 mm long, puberulent, early deciduous. Flowers sessile, creamy-white; calyx 5-lobed, 1.2–1.6 mm long, lightly appressed pubescent; corolla 5-lobed, 1.9–2.6 mm long, lightly appressed pubescent; stamen filaments 5.0–6.5 mm long; ovary glabrous, on a stipe to 0.4 mm long. Legumes light yellowish brown to dark brown, straight, flattened, oblong,

100–185 × 16–25 mm, cartilaginous, transversely striate, glabrous, eglandular, dehiscent; stipe to 15 mm long; apex acute to acuminate. Seeds uniseriate, no pulp, dark reddish brown, circular to nearly oblong, strongly flattened, 7.3–10.5 × 5.5–8.5 mm, smooth; pleurogram U-shaped, 2.2–3.5 mm across. Flowers: April–August.

Distribution. Open dry forest, dense thorn-scrub thickets, and dry rocky slopes below 1800 m elevation in the foothills and mountains of northeastern Mexico in the states of Coahuila, Guanajuato, Hidalgo, Nuevo León, Querétaro, San Luis Potosí, and Tamaulipas (Fig. 3).

Abundant in northeastern Mexico, *Acacia coulteri* is a common component of relatively dry forests and thorn-scrub thickets. Many of the collections are from roadsides and rocky pastures. This taxon is abundant in the states of Tamaulipas and San Luis Potosí, becoming less common to the south. *Acacia coulteri* is similar morphologically to *A. russelliana,* which is common in Sonora and parts of Sinaloa. *Acacia russelliana* is usually a small shrub or understory tree that rarely exceeds 4 m in height. *Acacia russelliana* has usually been considered as conspecific with *A. coulteri,* Britton and Rose (1928) being the only authors that recognized the two as distinct. Both of these taxa have leaves with fewer than 12 pinna pairs, pinnae with fewer than 35 leaflet pairs, petiolules that exceed 2.4 mm in length, and identical petiolar glands. Though similar in many traits, these taxa are easy to separate, *A. coulteri* having appressed pubescence on the lower leaflet surface, perianth, and the rachis and pinna rachises; *A. russelliana,* in contrast, being glabrous throughout.

The stipules of *Acacia coulteri* seedlings are decidedly spinescent, but are only weakly rigid after the first leaf stage, and become progressively smaller and less rigid on older plants (Vassal, 1972). Because this species lacks prickles and because of correlations of the cotyledonary petiole and shape, as well as certain other features, Vassal placed *A. coulteri* in his subgenus *Acacia,* a group roughly equivalent to Bentham's (1842) *Acacia* series *Gummiferae.* However, *A. coulteri* differs in some significant features from members of that series. Chief among them, the pollen grains of *A. coulteri* are porate, whereas those of other members of Bentham's series *Gummiferae* are colporate (Vassal, 1972).

Representative specimens. MEXICO. **Coahuila:** Cañon el Puerto, Sierra de Santa Rosa, *Villarreal, Vásquez, Gutíerrez & Urbina 5958* (TEX*). **Guanajuato:** between Aurora and Xichu, *McVaugh 14866* (MICH*); Mina de La Liga, *Ventura & López 8935* (MEXU*). **Hidalgo:** 10 km al NW de Zimapán, *González 2358* (DS, LL, MICH*). **Nuevo León:** jungles, N of Linares, *Clark 6819* (MO*); El Cercado, Santiago, *Hinton 24102* (NY*, TEX); Sierra de la Silla, *Pringle 2549* (A*, CM, F, G, GH, MO, NY, PH, UC, US, VT, WIS); N slope of La Silla, *White & Chatters 8* (GH, MICH*, TEX). **Querétaro:** 15 km al SE de Agua Zarca, *Rubio 1675* (MICH*, MO, TEX, WIS). **San Luis Potosí:** 10 mi. W of Tamuin on the Tampico–Valles hwy., *Crutchfield & Johnston 5401* (MICH*, TEX); Minas de San Rafael, *Purpus 5181* (F*, G, GH, MO, NY, UC, US); 16 km W of Tamuin, *Seigler, Clarke & Potgieter 13010* (EIU*, ILL). **Tamaulipas:** Ejido Los Angeles, *Diaz, Cedilla & Jiménez 484* (MEXU*); Camino a la mina El Berrinche, Victoria, *Jiménez 67* (MEXU*); 60 mi. N of Victoria, *Hitchcock & Stanford 6867* (CU, DS, F, GH, ILL*, ISC, MO, NY, UC, US); along route 70 about 3 mi. S of Victoria, *King 4498* (F, MICH, NY, TEX*,UC, US); 2 km WNW of Gómez Farías, *Martin 71C* (MICH, VT*); Puerto de la Angostura, km 658–60 between Victoria and Mante, *Moore, Jr. & Wood, Jr. 3629* (A*, BH, MICH, UC); Victoria, *Nilson 4421* (GH, NY, US*); 33 mi. N of Mante, *Seigler, Richardson & Thompson 11627* (EIU*, ILL).

13. Acacia russelliana (Britton & Rose) Lundell, Contr. Univ. Michigan Herb. 4: 7. 1940. *Senegalia russelliana* Britton & Rose, N. Amer. Fl. 23: 112. 1928. TYPE: Mexico. Sinaloa: vicinity of San Blas, 22 Mar. 1910, *J. N. Rose, P. C. Standley & P. G. Russell 13204* (holotype, US!; isotype, GH!, NY!).

Shrub or small tree to 8 m tall with bark dark gray, shallowly furrowed; twigs light brown to greenish brown, not flexuous, glabrous; short shoots absent. Leaves alternate, 60–140 mm long; stipules herbaceous, light brown, narrowly linear, to 2.5 × 0.4 mm near the base, glabrous, tardily deciduous; petiole adaxially shallowly grooved, 20–50 mm long, glabrous; petiolar gland solitary, located near the middle of the petiole to just below the lowest pinna pair, sessile, usually circular, 0.4–1.5 mm across, doughnut- to urn-shaped, glabrous, sometimes absent; rachis shallowly grooved adaxially, 30–90 mm long, glabrous, rarely a sessile, doughnut-shaped gland, 0.4–0.9 mm across, between the upper pinna pair; pinnae (2)4 to 11 pairs per leaf, 35–70 mm long, 5–12 mm between pinna pairs; petiolules 2.0–3.5 mm long; leaflets 18 to 34 pairs per pinna, opposite, 0.9–1.8 mm distance between leaflets, oblong, 4.0–7.5 × 1.3–1.8 mm, glabrous, lateral veins obvious with a midvein and 1 to 3 smaller veins from the base, base oblique, margins sometimes lightly ciliate, apex obtuse to broadly acute. Inflorescence a loosely flowered cylindrical spike 25–60 mm long, solitary (rarely 2 to 3) from the leaf axil, or rarely in terminal racemose clusters; peduncle 1–10 × 0.4–0.7 mm, glabrous; involucre absent; floral bracts linear, to 1 mm long,

glabrous, early deciduous. Flowers sessile, creamy-white; calyx 5-lobed, 1.0–1.4 mm long, glabrous; corolla 5-lobed, 1.7–2.5 mm long, glabrous; stamen filaments 5–7 mm long; ovary glabrous, on a stipe 2–3 mm long. Legumes greenish brown to dark brown, straight, flattened, oblong, 55–170 × 16–27 mm, cartilaginous, transversely striate, glabrous, eglandular, dehiscent; stipe to 15 mm long, apex acute to obtuse, sometimes beaked. Seeds uniseriate, no pulp, reddish brown, circular to oval, strongly flattened, 6.8–9.6 × 5.1–8.0 mm, smooth; pleurogram U-shaped, 2–3 mm across. Flowers: March–August.

Distribution. Dry, deciduous, tropical forests to thorn-scrub and desert-scrub vegetation, mostly on rocky slopes, from near sea level to about 700 m elevation in southwestern Sonora, and extreme northern Sinaloa, Mexico (Fig. 3).

A small shrub or understory tree not exceeding 8 m in height, *Acacia russelliana* is a common species of Sonora and parts of Sinaloa. Occurring at elevations below 700 m, it appears to be a common component of desert and thorn-scrub vegetation, and is also found as an understory tree in tropical deciduous forests.

Britton and Rose (1928) were the first to recognize this taxon, all previous and subsequent authors considering it to be conspecific with *Acacia coulteri*, a species of northeastern Mexico. It is easily distinguished from this taxon by being essentially glabrous; *A. coulteri*, in contrast, has leaves that are lightly pubescent beneath with appressed hairs, and the petiole and rachis are lightly puberulent, as are many other vegetative parts of the plant. Also, the perianth of most flowers of *A. coulteri* is puberulent, but it is glabrous in *A. russelliana*.

The only acacia species of this group found within the range of *Acacia russelliana* are *A. willardiana* and *A. millefolia*. Both taxa are easily separated from *A. russelliana*; *A. willardiana* by its extremely long, flattened petioles topped with 1 to 3 pairs of pinnae, and *A. millefolia* by the stalked gland with a bulbous apex between the upper pinna pair, the long, usually persistent stipules, and the missing petiolar gland.

It is quite probable that *Acacia russelliana* occasionally hybridizes with *A. willardiana* in thorn-scrub forests on the arid, rocky slopes at lower elevation in extreme northern Sinaloa, Mexico. The few specimens available suggest that the probable hybrid is similar to *A. willardiana* in being a small tree with exfoliating, papery bark, petioles that commonly exceed 100 mm in length, and pinnae with fewer than 26 pairs of leaflets that are relatively widely spaced. The proposed hybrid is similar to *A. russelliana* in having stipules to 2.5 mm long, petioles that are round in cross section and slightly adaxially grooved, a doughnut-shaped petiolar gland, and leaves with 2 to 11 pairs of pinnae. Few specimens are available for study [*Gentry 14337* (MICH, US), *Gentry 14420* (LL, MICH, US), *Gibson & Gibson 2101* (ARIZ, ASU), *Rose, Standley & Russell 13317* (US)]; field studies will be necessary to determine the status of these specimens.

Representative specimens. MEXICO. **Sinaloa:** vicinity of Culiacán, *Brandegee s.n.* (GH*, UC); Cerros de Navachiste about Bahía Topolobampo, *Gentry 14309* (US*); Cerros del Fuerte, 18–24 mi. N of Los Mochis, *Gentry 17635* (LL*); near Yacht Hotel Topolobampo, *Hastings & Turner 64-103* (SD*); vicinity of Culiacán, *Rose, Standley & Russell 14994* (US*). **Sonora:** 34 km E of Hermosillo on road to Sahuaripa, *Felger, Aronson & Shmida* 84-204 (SD, TEX*); foothills at S end of Sierra Libre, 12.3 mi. S of La Palma on hwy. 15, *Felger & Reichenbacher 85-1084* (TEX*); SW of Ures, *Felger 3000* (ARIZ, LL*); Río Sonora, 22.2 mi. by road E of Mex. 15 on road to Ures, *Felger 3628* (ARIZ*); Lake Mocuzari, *Johnson 191-77* (CAS*); Cerro Prieto, 8.6 mi. E of Navajoa, *Sanders, Guzy, Way, Charlton & McIntosh 4618* (NY*, TEX); summit of Cerro Prieto, 15 km E of Navajoa, *Sanders, Ballmer, Charlton, Clarke & Mayor 9286* (SD*), Río Mayo area, 9 mi. NW of Alamos, *Sanders, Ballmer, Charlton, Clarke & Mayor 9440* (SD*, TEX); Navajoa, summit of Cerro Masiaca, *Sanders, Devender, Devender, Meyer & Pitzer 12767* (CAS*, MO, TEX, WIS); Alamos, Cerro La Luna, *Sanders, Friedman, Spenger & Kossack 13260* (CAS*, MO, TEX); at crossing of Río Sonora, 23 mi. NE of El Sacatón, 19 Sep 1934, *Shreve 6703* (GH*, MICH, MO); Cerro Prieto, 15 km al E de Navajoa, *Tenorio, Romero, Ignacio & Davila 10202* (F, CAS, MO, NY*); near the Mirador, Alamos, *Devender & Lindquist 94-828* (NY*); crossing the Río Sonora, 13 mi. S of Ures, *Wiggins 7320* (A, DS, F, MICH, UC, US*); 15 mi. S of La Palma, between Hermosillo and Guaymas, *Wiggins & Rollins 228* (A, LL, DS*, MICH, MO, NY, UC).

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A REVIEW OF GAMOSEPALY IN THE BRASSICACEAE AND A REVISION OF *DESIDERIA*, WITH A CRITICAL EVALUATION OF RELATED GENERA[1]

Ihsan A. Al-Shehbaz[2]

ABSTRACT

Gamosepaly is reported in 12 genera of the Brassicaceae and is considered to have evolved independently as many times. It is concluded that gamosepaly is not a useful character for the circumscription of genera in the family. The boundaries of *Desideria* and several other genera are critically evaluated, and a taxonomic revision of *Desideria* is presented. A new name (*D. haranensis*), eight new combinations (*D. baiogionensis, D. flabellata, D. himalayensis, D. incana, D. linearis, D. prolifera, D. pumila,* and *D. stewartii*), and eight new synonyms (*Christolea karakorumensis, C. pinnatifida, C. scaposa, Desideria pamirica, Ermania bifaria, E. kachoori, E. kashmiriana,* and *E. parkeri*) are proposed. As herein delimited, *Christolea* consists of only two species; *Ermania* is reduced to synonymy of *Melanidion,* and *Ermaniopsis* and *Oreoblastus* are reduced to synonymy of *Desideria.* The relationship and distinguishing characters of *Desideria, Christolea, Ermania, Eurycarpus, Leiospora, Melanidion,* and *Solmslaubachia* are discussed.

Key words: Brassicaceae, *Christolea, Desideria, Ermania, Eurycarpus,* gamosepaly, *Leiospora, Melanidion, Solmslaubachia.*

During work on the Brassicaceae (Cruciferae) for the *Flora of China, Flora of Nepal,* and *Flora of Kazakstan,* it became evident that the limits of several Himalayan and Central Asian genera needed critical evaluation, and the nomenclature of many species and infraspecific taxa needed adjustments. The genera addressed in the present paper are *Christolea* Cambess., *Desideria* Pamp., *Ermania* Cham. ex Botsch., *Ermaniopsis* H. Hara, *Eurycarpus* Botsch., *Leiospora* (C. A. Mey.) Dvořák, *Melanidion* Greene, *Oreoblastus* Sus-lova, and *Solmslaubachia* Muschl. They exhibit overlapping similarities in several characters, and their limits have often been confused.

Because *Desideria* was based on a species with a gamosepalous calyx, a review of gamosepaly in the Brassicaceae is presented to determine whether or not this character alone is sufficient to establish genera. The study led to the revision of *Desideria* and also critically evaluated the limits of several presumably related genera.

GAMOSEPALY IN THE BRASSICACEAE

Gamosepaly has been reported in at least 12 genera of the Brassicaceae from Asia and South America. It was first reported by Oliver (1893) in *Braya uniflora* Hook. f. & Thomson. Hooker and Thomson (1861) and Hooker and Anderson (1872) did not report gamosepaly in the species even though the type collection has all flowers and fruits with persistent, united sepals. Schulz (1924) transferred the species to the monotypic *Pycnoplinthus* O. E. Schulz, a genus restricted to China and Kashmir (Jafri, 1973; Kuan, 1987; Hajra et al., 1993).

Desideria mirabilis Pamp. (China, Kashmir, Tajikistan) is the second species reported to have a gamosepalous calyx, and it too was placed in a monotypic genus (Pampanini, 1926, 1930). Hedge (1968b) described *Sisymbrium gamosepalum* Hedge and *Arabidopsis gamosepala* Hedge, both of which are endemic to Afghanistan, but the latter species was transferred by Al-Shehbaz and O'Kane (1997) to *Neotorularia* Hedge & J. Léonard. *Sisymbrium* L. (ca. 50 species; Al-Shehbaz, unpublished) is represented by indigenous species on all continents except Australia and Antarctica (Al-Shehbaz, 1988), whereas *Neotorularia* includes about 15 species distributed primarily in Central Asia and the Middle East (Al-Shehbaz, unpub-

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lished). *Sisymbrium* and *Neotorularia* each includes only a single species with a gamosepalous calyx.

Two additional species of *Desideria*, *D. pamirica* from Tajikistan (Suslova, 1973) and *D. nepalensis* from Nepal (Hara, 1975), were described with a gamosepalous calyx. The reports of gamosepaly in *Christolea scaposa* by Jafri (1973), *C. karakorumensis* by Wu and An (1994), and *D. pamirica* are shown in the present study to be erroneously based on plants of *D. mirabilis*.

Gamosepaly was first reported from South America by Al-Shehbaz (1990b) in *Brayopsis* Gilg & Muschl., a genus of six species of which only *B. gamosepala* Al-Shehbaz (Bolivia) has united sepals. An examination of other South American species revealed gamosepaly in *Catadysia rosulans* O. E. Schulz (Appel, pers. comm.) and *Eudema friesii* O. E. Schulz (Martínez-Laborde, pers. comm.). *Eudema* Humb. & Bonpl. includes six species distributed from Ecuador into Argentina and Chile (Al-Shehbaz, 1990a), of which only *E. friesii* has a gamosepalous calyx, whereas *Catadysia* O. E. Schulz is a monotypic genus endemic to Peru (Schulz, 1929, 1936).

Gamosepaly has recently been discovered in one of six species of the Himalayan *Pegaeophyton* Hayek & Hand.-Mazz., *P. watsonii* Al-Shehbaz of Sikkim (Al-Shehbaz, 2000a), and in one of six species of the Himalayan and Central Asian *Phaeonychium* O. E. Schulz, *P. jafrii* Al-Shehbaz (Al-Shehbaz, 2000b), although the type collection of the latter has plants with free and united sepals. *Solmslaubachia xerophyta* (W. W. Sm.) Comber (China) also has calyces with either free or completely united sepals, whereas *S. gamosepala* Al-Shehbaz & G. Yang (China), which is known only from the type collection, has united sepals (Al-Shehbaz & Yang, 2000b).

At least one of the approximately 150 species of *Erysimum* L., *E. siliculosum* (M. Bieb.) DC., has a gamosepalous calyx. The species was previously recognized in *Syrenia* Andrz., a genus that I place in the synonymy of *Erysimum*. It is likely that some of the species related to *E. siliculosum* also have gamosepalous calyces, but I have not examined adequate material of those.

In all four species of *Pugionium* Gaertn. (northern China, Mongolia, and adjacent Siberian Russia) the sepals are connate. As the fruit develops, the calyx ruptures basally along the lines of sepal connation.

Finally, the genus *Gamosepalum* Hausskn. was initially thought to have a gamosepalous calyx (Schulz, 1927b, 1936). However, careful examination of its component species revealed that the sepals are free, but they appear connate because of interlocking stellate trichomes (Dudley, 1964).

In conclusion, two generalizations can be made regarding gamosepaly. First, the union of sepals evolved independently several times in the Brassicaceae. It is not known whether it evolved one or more times within *Desideria*, but a phylogenetic study based on molecular data should reveal that. With the critical examination of more genera of Brassicaceae, it is likely that more species with gamosepalous calyces will be found. Second, gamosepaly alone cannot be used to define the boundaries of genera because it occurs in several genera in which the majority of species have free sepals. Therefore, in the present delimitation of *Desideria* gamosepaly is ignored as a generic character, and the overall similarities and relationships of species are emphasized.

Nothing is known about the inheritance of gamosepaly in the family, but the occurrence of plants with free and united sepals in the same population of *Phaeonychium jafrii* is a good lead for conducting a simple experiment to test the genetic basis of this character.

Generic Relationships and Circumscriptions

DESIDERIA

Pampanini (1926) established the monotypic *Desideria* solely on the basis of having a gamosepalous calyx. Although he indicated that *D. mirabilis* resembles what was then known as *Cheiranthus himalayensis* Cambess., Schulz (1927a, 1936), Botschantsev (1955, 1956), and Jafri (1955) regarded gamosepaly as an anomaly and reduced *D. mirabilis* to synonymy of *C. himalayensis*, a species that Schulz and Botschantsev assigned to *Ermania* and Jafri to *Christolea*. However, these authors overlooked the significant features (see below) that distinguish these two species. With the description of two additional species in *Desideria* (Suslova, 1973; Hara, 1975), the genus was recognized as distinct in subsequent floristic works (e.g., Czerepanov, 1995; Hara, 1979; Pachomova, 1974; Yunussov, 1978), and it remained to be delimited primarily on the basis of having a gamosepalous calyx.

A critical evaluation of all genera related to *Desideria* in this paper leads to the conclusion that the genus should include 6 of the 10 species treated in *Ermania* by Schulz (1936), 8 of the 10 species recognized in *Ermania* by Botschantsev (1955), and 5 of the 13 species assigned to *Christolea* by Jafri (1955). The species recognized by these authors in *Christolea* or *Ermania* and excluded from *Desideria* in the present account are: *Par-*

rya villosa Maxim. and *Cheiranthus albiflorus* T. Anderson, which now belong to *Phaeonychium* (Al-Shehbaz, 2000b); *Draba parryoides* Cham. and *Melanidion boreale* E. L. Greene, which are assigned to *Melanidion* (see below); *Christolea crassifolia* Cambess., which is retained in *Christolea*; and *Parrya lanuginosa* Hook. f. & Thomson, which is placed in *Eurycarpus* Botsch. (Al-Shehbaz & Yang, 2000a).

As herein delimited, *Desideria* consists of 11 Himalayan, Chinese, and Central Asian species characterized by having well-defined basal rosettes, slender and rhizome-like caudices, orbicular or flabellate to broadly ovate or obovate, often dentate and palmately veined basal leaves, simple and/or forked trichomes, linear to linear-lanceolate latiseptate fruits rectangular in cross section, nontorulose and strongly veined valves with distinct marginal veins, valve apices united with the replum, often obsolete styles, 2-lobed stigmas, and accumbent cotyledons. A combination of fruits rectangular in cross section, valves with prominent marginal veins, valve apices united with the replum, often obsolete styles, and dentate leaves often palmately veined readily distinguish *Desideria* from the other genera discussed in this paper.

CHRISTOLEA, ERMANIA, AND *MELANIDION*

In his description of *Draba parryoides,* Chamisso (1831: 533) stated, "DRABA? *parryoides* n. sp. vel potius novum genus e solo fructu, deficiente flore, haud rite definiendum. *Drabis* dolichocarpis subjungimus pro tempore plantam aliquando fors jure meritoque nomine inventoris ERMANIAM *parryoidem* slutandam." Several workers (e.g., Schulz, 1936; Botschantsev, 1955; Hedge, 1968a; Suslova, 1972; Ovczinnikov & Yunussov, 1978; Greuter et al., 1993) considered the above statement as a valid publication of the genus *Ermania,* while others (e.g., Jafri, 1955, 1973; Jurtsev, 1975; Berkutenko, 1988; Czerepanov, 1995) did not. According to Article 34 of the *Code* (Greuter et al., 2000), Chamisso's statement does not constitute valid publication of the genus. Despite Schulz's (1936) detailed description of *Ermania,* it was in German, and the genus remained invalidly published until Botschantsev (1956) provided the Latin diagnosis. Therefore, all transfers to *Ermania* proposed by Schulz (1927a, 1933a, b, c) and Botschantsev (1955) remained invalid. As it is presently delimited, *Ermania* includes only *E. parryoides* (Cham.) Botsch., the generic type, and all other species assigned to it belong to other genera. *Ermania* does not occur in the Himalayas and Central Asia and, therefore, regardless of the interpretation of its effective date of valid publication, it does not affect the nomenclature of the unrelated taxa herein placed in *Desideria.*

Although superficially resembling some species of *Desideria* and *Christolea, Ermania parryoides* is most closely related to *Melanidion boreale* E. L. Greene. Both species have Arctic and subarctic distribution (the Russian Far East for the former and Alaska, Yukon, and Northwest Territories for the latter) and are similar in habit, foliage, pubescence, flowers, and fruit morphology. Hultén (1945) was the first to point out this close relationship, and he transferred *M. boreale* to *Ermania,* but his transfer was illegitimate because *Ermania* was invalidly published. The principal difference between these species is that *E. parryoides* has latiseptate fruits (flattened parallel to the septum) and *M. boreale* has angustiseptate fruits (flattened at a right angle to the septum), but this difference is not as significant as once thought because there are many genera of the Brassicaceae with both fruit types. Drury and Rollins (1952) and Rollins (1993) reduced *Melanidion* to synonymy of *Smelowskia* C. A. Mey., but their circumscription of the North American *Smelowskia* was so broad that some of the species recognized are doubtfully congeneric. If *M. boreale* and *E. parryoides* were kept in a genus distinct from *Smelowskia,* as I presently support, then *Ermania* would have to be abandoned and the earlier published *Melanidion* recognized. These two species will be dealt with in a subsequent publication.

In his original description of *Cheiranthus himalayensis* and *Christolea crassifolia,* Cambessèdes (1844) did not indicate anything about their relationship or similarities to each other. However, Jafri (1955) placed them and several other species in *Christolea* and adopted a broad generic concept that included species presently assigned to the genera *Christolea, Desideria, Eurycarpus, Melanidion, Parrya* R. Br., and *Phaeonychium.* With such a broad delimitation, several additional genera, especially *Pegaeophyton* and *Pycnoplinthus,* could have easily been included in *Christolea* without expanding the generic limits any further. Unfortunately, Jafri's delimitation of *Christolea* was closely followed in some of the more recent floras (e.g., An, 1987, 1995; Hajra et al., 1993; Huang, 1997b; Kuan, 1985).

Ovczinnikov and Yunussov (1978) also adopted a rather broad concept of *Ermania* by including *Christolea* and *Oreoblastus* as sections. These authors differed from Jafri (1955) primarily in their decision about the effective date of valid publication of *Ermania.* In my opinion, their vastly het-

erogeneous generic circumscriptions of *Christolea* or *Ermania* are unacceptable. *Christolea* consists of two species, the Himalayan *C. crassifolia* and the Chinese endemic *C. niyaensis* Z. X. An, and it differs from *Melanidion* (including *Ermania*) by having many-leaved stems, nonrosulate lower leaves, exclusively simple trichomes, incumbent cotyledons, apiculate anthers, and transversely oriented seeds. By contrast, *Melanidion* has leafless stems, well-developed basal rosettes, dendritic trichomes mixed with simple ones, accumbent or obliquely accumbent cotyledons, obtuse anthers, and longitudinally oriented seeds.

Desideria differs from both *Melanidion* and *Christolea* by having fruits rectangular in cross section, valves with prominent marginal veins, and valve apices united with the replum. From *Christolea*, *Desideria* differs by having a well-developed basal rosette, usually leafless stems, slender and rhizome-like caudices, often palmately veined leaves, nontorulose fruits, longitudinally oriented biseriate seeds, and accumbent cotyledons. By contrast, *Christolea* has nonrosulate lower leaves, leafy stems, compact and woody caudices, pinnately veined leaves, strongly torulose fruits, transversely oriented uniseriate seeds, and incumbent cotyledons. The Himalayan and Central Asian *Desideria* also differs from the Arctic and subarctic *Melanidion* by lacking the dendritic trichomes and having sessile 2-lobed stigmas, smooth fruits, biseriate seeds, and toothed nectaries lacking the median glands. *Melanidion* has dendritic trichomes, entire and capitate stigmas on distinct styles, torulose fruits, uniseriate or rarely subbiseriate seeds, and annular nectaries with well-developed median glands. A comparison of *Desideria* with the presumably related genera is summarized in Table 1.

EURYCARPUS

In establishing the genus *Eurycarpus*, Botschantsev (1955) separated it from *Ermania* by having biseriate instead of uniseriate seeds, broadly lanceolate instead of linear fruits, entire instead of dentate leaves, and leafless instead of leafy scapes. However, he probably compared only the type species of both genera because most of the differences above do not hold if one compares *Eurycarpus* with the ten species Botschantsev recognized in *Ermania*. As indicated above, eight of Botschantsev's ten species of *Ermania* are presently assigned to *Desideria*. A comparison of *Desideria* with *Eurycarpus* (two species) sensu Al-Shehbaz and Yang (2000a) shows that the latter differs by having entire and pinnately veined leaves, obscurely veined valves without marginal veins, broadly lanceolate to oblong fruits narrowly elliptic in cross section, well-defined subconical styles, and minute, entire stigmas much narrower than the style. By contrast, *Desideria* almost always has dentate, palmately veined leaves, prominently veined valves with well-developed marginal veins, linear to linear-lanceolate fruits rectangular in cross section, obscurely differentiated or cylindric styles, and distinct, often 2-lobed stigmas as broad as the style.

OREOBLASTUS

Although Jafri (1973) admitted the artificiality of his delimitation of *Christolea*, he (p. 155) correctly stated that, "Even if, *Christolea* Camb. (s. str.) and *Ermania* Cham. ex [Botschantsev] Schulz (s. str.) are considered as separate genera, there can be no doubt that *Oreoblastus* Suslova is congeneric with *Desideria* Pamp., where most of our species would go."

Suslova (1972) separated *Oreoblastus* from *Desideria* by having free instead of united sepals, a deciduous instead of persistent calyx, and septate instead of eseptate fruits. However, she must have overlooked the persistent calyx in several specimens that she annotated as *Oreoblastus*, and the holotype of her *D. pamirica* (Suslova, 1973) has septate instead of eseptate fruits, though the septa are perforated but never lacking. Except for having free instead of united sepals, *Oreoblastus* is indistinguishable from *Desideria*. As indicated above, sepal connation alone is insufficient for the establishment of genera and, therefore, *Oreoblastus* is reduced herein to synonymy of the earlier published *Desideria*.

ERMANIOPSIS

The presence vs. absence of a tooth on the median stamens was considered by some (e.g., Schulz, 1936; Hara, 1974; Golubkova, 1976) as an important generic character. In my opinion, this feature alone does not justify the segregation of genera. Toothed and toothless filaments are found in *Dontostemon* Andrz. ex C. A. Mey. (Al-Shehbaz & Ohba, 2000), whereas winged or wingless, toothed or toothless, and appendaged or unappendaged filaments are found in *Alyssum* L. (Al-Shehbaz, 1987; Dudley, 1964).

Although Hara (1974) provided a detailed discussion to distinguish *Ermaniopsis* from *Ermania* and related genera, the single character that sets *Ermaniopsis* apart is the presence of a lateral tooth on the filaments of median stamens. On the basis of all other characters, *Ermaniopsis pumila* H. Hara

Table 1. Comparison of *Desideria*, *Christolea*, *Eurycarpus*, *Leiospora*, *Melanidion*, and *Solmslaubachia*.

	Desideria	*Christolea*	*Eurycarpus*	*Leiospora*	*Melanidion*	*Solmslaubachia*
No. of species	11	2	2	6	2	9
Trichomes	simple and/or forked	simple	simple and/or forked	simple and/or forked or absent	dendritic with some simple and forked	absent or simple
Basal leaves	rosulate	not rosulate	rosulate	rosulate	rosulate	rosulate
Leaf margin	dentate	dentate	entire	entire or dentate	dentate	entire
Leaf venation	palmate	pinnate	pinnate	pinnate	palmate	pinnate
Cauline leaves	present or sometimes absent	present	absent	absent	present	mostly absent
Flowers	solitary or in racemes	in racemes	in racemes	solitary or in racemes	in raceme	solitary or rarely in racemes
Sepals	equal, nonsaccate	equal, nonsaccate	equal, nonsaccate	unequal, inner pair saccate	equal, nonsaccate	equal, nonsaccate
Anther apex	obtuse	apiculate	obtuse	obtuse	apiculate	obtuse
Anther shape	ovate to oblong	oblong	oblong	linear	oblong	linear-oblong
Median nectar glands	present or absent	present	present	absent	present	absent
Fruit attachment to pedicel	readily detached from pedicel	persistent on pedicel	persistent on pedicel	readily detached from pedicel	persistent on pedicel	readily detached from pedicel
Fruit valve	smooth	torulose	smooth	torulose	smooth	smooth
Fruit in cross section	rectangular	transversely narrowly oblong or elliptic	transversely narrowly elliptic	rectangular	transversely narrowly oblong or elliptic	rectangular
Fruit marginal veins	prominent	obscure	obscure	prominent	obscure or prominent	prominent
Valve apex and replum	adnate	readily detached	readily detached	adnate	readily detached	adnate
Style	obsolete	obsolete or distinct	distinct	absent	distinct	obsolete or distinct
Stigma	slightly 2-lobed	entire or slightly 2-lobed	entire	strongly 2-lobed	entire	entire or slightly 2-lobed
Stigma lobes	not decurrent	not decurrent	NA	strongly decurrent	NA	not decurrent
Seed arrangement	uniseriate or biseriate	uniseriate	biseriate	uniseriate or biseriate	uniseriate	uniseriate or biseriate
Seed margin	wingless, not margined	wingless, not margined	wingless, not margined	winged or margined	wingless, not margined	wingless, not margined
Cotyledons	accumbent	incumbent	accumbent or incumbent	accumbent	obliquely accumbent or obliquely incumbent	accumbent

is perfectly at home in *Desideria*. In fact, Hara indicated that *E. pumila* resembles *Desideria* (as *Parrya*) *pumila* in vegetative characters. A close examination of flower and fruit characters clearly shows that the two species are congeneric, and *Ermaniopsis* is reduced herein to synonymy of *Desideria*. Unfortunately, both species have the same epithet, and *E. pumila* is named hereafter as *D. haranensis*. The median filaments of both *D. pumila* and *D. haranensis* are dilated, and only the latter species shows a minute to prominent tooth on the median staminal filaments.

SOLMSLAUBACHIA AND *LEIOSPORA*

On the basis of fruit morphology, *Desideria* is most closely related to *Solmslaubachia* (9 spp.: 8 endemic to China and 1 extending also into Bhutan and Sikkim) and the Central Asian and Himalayan *Leiospora* (6 spp.). All three genera have fruits readily detached from the pedicel, and their valves are adnate apically to the replum. Upon maturity, the fruit falls off the plant and its apex remains tardily dehiscent. The three genera also have obsolete or no styles, and their fruit valves are strongly angled at the margins and completely conceal the replum. These combinations of characters are not found in any Himalayan or Central Asian genera of the Brassicaceae.

Desideria is easily separated from *Solmslaubachia* by having palmately veined leaves apically 3- to 9(to 11)-toothed, ovate to oblong anthers often less than 1 mm long, and forked trichomes sometimes mixed with simple ones. By contrast, *Solmslaubachia* has entire, pinnately veined leaves, linear-oblong anthers more than 1 mm long, and exclusively simple trichomes.

Desideria is readily distinguished from *Leiospora* by having wingless seeds, equal sepals with the lateral pair nonsaccate, palmately veined leaves apically 3- to 9(to 11)-toothed, oblong-linear anthers 0.4–1(–1.6) mm long, and capitate, slightly 2-lobed stigmas with neither decurrent nor connivent lobes. *Leiospora* often has winged or margined seeds, unequal sepals with the lateral pair strongly saccate, entire or marginally dentate leaves, linear anthers 2.5–3 mm long, and conical, prominently 2-lobed stigmas with connivent, decurrent lobes.

TAXONOMIC TREATMENT

Desideria Pamp., Bull. Soc. Bot. Ital. 1926: 111. 1926. TYPE: *Desideria mirabilis* Pamp.

Ermaniopsis H. Hara, J. Jap. Bot. 49: 198. 1974. TYPE: *Ermaniopsis pumila* H. Hara.

Oreoblastus Suslova, Bot. Zhurn. (Moscow & Leningrad) 57: 648. 1972. TYPE: *Oreoblastus flabellatus* (Regel) Suslova.

Herbs perennial, with a slender, often many-branched, rhizome-like caudex often covered with remains of basal rosettes. Trichomes simple and/or mixed with short-stalked forked ones. Stems simple, leafy or leafless, sometimes absent. Basal leaves petiolate, rosulate, simple, 3- to 9(to 11)-toothed, often palmately veined, persisting whole or only petioles persistent. Cauline leaves similar to basal ones, entire or toothed, subsessile or petiolate, or absent. Racemes 3- to 30-flowered, dense or lax, bracteate throughout or ebracteate, corymbose, elongated or not elongated in fruit, sometimes flowers solitary on pedicels originating from basal rosette. Sepals ovate to oblong, free or united, deciduous or persistent, erect, equal, base of inner pair not saccate, margins membranous. Petals purple, purple-green, or rarely white, sometimes yellowish at base of blade; blade obovate to spatulate, apex obtuse to subemarginate; claw strongly differentiated from blade, subequaling or longer than sepals. Stamens 6, erect, tetradynamous; filaments wingless or rarely winged, toothless or rarely toothed, free, dilated at base; anthers ovate to oblong, not apiculate at apex. Nectar glands 2 and lateral, or 1 and confluent outside bases of all stamens; median nectaries present or absent. Ovules 10 to 70 per ovary. Fruit dehiscent siliques, linear to lanceolate, latiseptate, rectangular in cross section, not inflated, sessile; valves papery, with a prominent midvein and distinct marginal veins, glabrous or pilose, smooth, adnate with replum at fruit apex; replum rounded, often concealed by valve margin; septum complete, perforated, or reduced to a rim, membranous, translucent, veinless, rarely absent; style obsolete; stigma capitate, slightly 2-lobed. Seeds uniseriate or biseriate, wingless, oblong to ovate, often flattened; seed coat obscurely reticulate, not mucilaginous when wetted; cotyledons accumbent.

Eleven species: Himalayas, western China, and adjacent central Asia.

KEY TO THE SPECIES OF *DESIDERIA*

1a. Sepals united, persistent till or after fruit dehiscence; septum absent or reduced to a rim.
  2a. Petals 11–13 × 5–6 mm; calyx 5–6 mm long; flowers 2–4, appearing solitary; Nepal ..... 11. *D. nepalensis*
  2b. Petals and calyx smaller; flowers more than 4, in distinct racemes; China, Kashmir, Tajikistan ..... 10. *D. mirabilis*

1b. Sepals free, caducous or rarely persisting till about fruit maturity; septum complete or rarely perforated apically.
3a. Flowers solitary from a basal rosette.
4a. Fruit ovate to broadly lanceolate, 6–9 mm wide, prominently reticulate veined ... 9. *D. baiogionensis*
4b. Fruit linear to linear-lanceolate, 2–5 mm wide, obscurely veined.
5a. Leaf trichomes forked and simple; replum retrorsely pilose; valves glabrous; sepals 3–4 mm long; petals 6–8 mm long ..... 8. *D. pumila*
5b. Leaf trichomes exclusively simple; replum and valves pilose to villous; sepals 6–7 mm long; petals 11–14 mm long ..... 7. *D. prolifera*
3b. Flowers (3 to)6 to 30 in a raceme.
6a. Racemes bracteate throughout.
7a. Stem and pedicel trichomes forked ..... 2. *D. stewartii*
7b. Stem and pedicel trichomes exclusively simple or absent.
8a. Fruit lanceolate to linear-lanceolate, (3–)4–6 mm wide; petals (6–)6.5–8 × 3–4 mm; seeds biseriate, (1.5–)1.8–2(–2.3) × 1–1.4 mm ..... 1. *D. himalayensis*
8b. Fruit linear, (0.8–)1–1.7(–2) mm wide; petals 4–5(–5.5) × 1.5–2.5 mm; seeds uniseriate, 0.8–1.1 × 0.5–0.8 mm ..... 3. *D. linearis*
6b. Racemes ebracteate.
9a. Filaments flattened, subapically toothed; petals 6.5–8 mm long; leaf trichomes minutely forked, mixed with short simple ones ..... 6. *D. haranensis*
9b. Filaments terete, toothless; petals 11–18 mm long; leaf trichomes either exclusively simple or distinctly forked.
10a. Plants canescent; leaf trichomes almost exclusively branched; leaves 3(to 5)-toothed ..... 5. *D. incana*
10b. Plants greenish; leaf trichomes exclusively simple, to 1.5 mm long; leaves (3 to)5- to 9(to 11)-toothed ..... 4. *D. flabellata*

1. Desideria himalayensis (Cambess.) Al-Shehbaz, comb. nov. Basionym: *Cheiranthus himalayensis* Cambess., in Jacquemont, Voy. Inde 4: 14. 1844. *Ermania himalayensis* (Cambess.) O. E. Schulz, Notizbl. Bot. Gart. Berlin-Dahlem 9: 1080. 1927. *Oreoblastus himalayensis* (Cambess.) Suslova, Bot. Zhurn. (Moscow & Leningrad) 57: 652. 1972. TYPE: [W Tibet.] "In declivitate orientali jugi vulgò Kioubrunghauti in Tartariâ sinensi," *Victor Jacquemont 1782* (holotype, P!; isotypes, K!, P!).

Plants 4–20 cm tall, densely pilose throughout to subglabrous. Trichomes simple, to 1.5 mm long. Stems simple, pilose or glabrous. Basal leaves not fleshy, pilose or glabrous, persistent; petiole 0.4–1.6(–3) cm long, not ciliate; leaf blade broadly obovate to spatulate, 4–14 × 3–9 mm, base cuneate to attenuate, margins (3 to)5-toothed, apex acute. Stem leaves similar to basal or linear to lanceolate, 5–17 × 1–4 mm, often entire, short petiolate to subsessile. Racemes 6- to 25-flowered, bracteate throughout; bracts similar to stem leaves but smaller, sometimes adnate to pedicel. Fruiting pedicels ascending, straight or curved, 3–10 mm long, pilose or glabrous. Sepals free, oblong, 3–4 × 1.2–1.5 mm, caducous, pilose or with a terminal tuft of hairs, base not saccate, margins membranous. Petals purple or lilac with yellowish center, broadly spatulate, (6–)6.5–8 × 3–4 mm, apex subemarginate; claw 3–4 mm long. Filaments white, slightly dilated at base, median pairs 3–4 mm long, lateral pair 2–4 mm long; anthers ovate, ca. 0.6 mm long. Ovules 7 to 12 per locule. Fruit lanceolate to linear-lanceolate, (1.7–)2–3.5(–4) cm × (3–)4–6 mm, strongly flattened; valves pilose or glabrous, distinctly veined; septum complete, membranous; style obsolete; stigma 2-lobed. Seeds brown, ovate, (1.5–)1.8–2(–2.3) × 1–1.4 mm, biseriate, minutely reticulate.

Phenology. Flowering June through August. Fruiting July to mid October.

Habitat and distribution. Alpine tundra, open hills, sandstone scree; 4300–5300 m. China (Qinghai, Xizang), India, Kashmir, Nepal.

Selected specimens examined. CHINA. **Qinghai:** Leixie-wu-den, *Wu, Huang & Yang K-890* (HNWP). **Xizang:** Baingoin Xian, Whale Lake, *Wu, Ohba, Wu & Fei 4075* (KUN, MO, TI); E of Moincer, 31°14′N, 80°56′E, *G. & S. Miehe 9643/17* (GOET, MO); NW Tibet, 34°55′N, 82°24′E, *Pike 842* (K); Aksu, *Deasy 92* (BM); Shuang Ho Xian, *Qinghai-Xizang Team 12009* (PE); Ritu Xian, *Qinghai-Xizang Team 76-9061* (KUN, PE), *Li Bosheng & Zhang Du 10980* (PE). INDIA. **Punjab:** Lahul, Kangra, Bara Lach La, *Koelz 6738* (GH). KASHMIR. Harnag, Upper Lidder valley, *Stewart 9349* (B, G, K, MO). NEPAL. Dhaulagiri Himal, hidden valley, between Dhampus pass and French pass, *Wald 65* (BM); Thorong La, Marsyandi Valley, *McBeath 1486* (E); Naurgaon, Marsyandi, *McBeath 1406* (E); Annapurna Himal, between N Annapurna Glacier base camp and top of N Annapurna Glacier icefall, 170–175 km N of Pokhara, *Komarkova 18* (GH).

Desideria himalayensis is most frequently confused with *D. linearis*, and some authors (e.g., Jafri, 1955) considered them to be conspecific. Three

collections of *Desideria linearis* (*Lyon 44, Stainton 3055, Stainton 3241*) were cited by Jafri (1973) as *Christolea himalayensis*, and the first two were listed by Hedge (1968a) as *Ermania himalayensis*. The two species can be readily separated by petal size, fruit width, and seed arrangement and size (see key). Mixed collections of the two species (e.g., *Koelz 6738*) are not uncommon, but no intermediates have been found. Both species can be distinguished from the related *D. stewartii* by having leaf and stem trichomes exclusively simple instead of forked.

Desideria himalayensis was reported (as *Christolea*) from Xinjiang by An (1995), but I have not seen any material from that part of China. One collection (*Polunin, Sykes & Williams 37*) was cited by Hara (1979) as this species, but this collection clearly belongs to *Desideria linearis*.

Desideria himalayensis was erroneously illustrated in Jafri (1973) with ebracteate inflorescences. It is likely that the plant illustrated belongs to *D. flabellata*, a species that occurs in bordering Afghanistan, China, Kyrgyzstan, and Tajikistan but is not yet reported from Kashmir. *Desideria himalayensis*, *D. stewartii*, and *D. linearis* are the only three species of *Desideria* that consistently have racemes bracteate throughout.

2. Desideria stewartii (T. Anderson) Al-Shehbaz, comb. nov. Basionym: *Cheiranthus stewartii* T. Anderson, in J. D. Hooker, Fl. Brit. India 1: 132. 1872. *Ermania stewartii* (T. Anderson) O. E. Schulz, Bot. Jahrb. Syst. 66: 98. 1933. *Christolea stewartii* (T. Anderson) Jafri, Notes Roy. Bot. Gard. Edinburgh 22: 53. 1955. *Oreoblastus stewartii* (T. Anderson) Suslova, Bot. Zhurn. (Moscow & Leningrad) 57: 653. 1972. TYPE: Kashmir. Ladak, 15,000–16,500 ft., *J. L. Stewart s.n.* (holotype, K!; isotype, E!).

Plants 8–20 cm tall, densely pilose. Trichomes stalked forked, rarely some simple near the stem base. Stems simple, pilose or glabrous. Basal leaves subfleshy, pilose, persistent; petiole 2–10 mm long, not ciliate; leaf blade broadly obovate to spatulate, 2–15 × 2–10 mm, base cuneate to attenuate, margins 3- to 5-toothed or subentire, apex acute. Stem leaves similar to basal or linear to lanceolate, often entire. Racemes 8- to 15-flowered, bracteate throughout; bracts similar to stem leaves but smaller, often adnate to pedicel. Fruiting pedicels ascending, straight or slightly curved, 4–12 mm long, pilose. Flowers not seen. Ovules 7 to 12 per locule. Fruit lanceolate to lanceolate-linear, 1.7–3.5 cm × 3–5 mm, strongly flattened; valves pilose or glabrous, distinctly veined; septum complete, membranous; style obsolete; stigma 2-lobed. Seeds brown, ovate, 1.4–2.2 × 0.8–1.1 mm, biseriate, minutely reticulate.

Phenology. Flowering unknown. Fruiting in August.

Habitat and distribution. Scree slopes; 4100–5000 m. China (Xizang), India, Kashmir.

Selected specimens examined. CHINA. **Xizang:** Ali, Geji, *Qinghai-Tibet Team 76-8652* (PE). INDIA. **Punjab:** Bara Lacha Pass, Lahul, *Cooper 5490* (E). **Himachal Pradesh:** Zingzingbar, *McBeath 2105* (E).

Desideria stewartii is a very rare species known thus far from the few collections cited above. Reports of the species from China (Kuan, 1985; An, 1987) are most likely based on misidentified plants of *D. himalayensis*.

Jafri (1973) doubted the distinction of *Desideria stewartii* from *D. himalayensis* (both as *Christolea*), and he confused the limits of the two species by citing one collection, *Stewart 9349*, under the former instead of the latter species. I have not seen any flowering material of the species, and the description of the flowers by Jafri (1973), which was followed by Hajra et al. (1993) and An (1987), was almost certainly based on a small flowering branch of *D. linearis* mounted on the holotype sheet of *D. stewartii*.

In overall aspects of foliage and fruit, *Desideria stewartii* most closely resembles *D. himalayensis*. However, *D. stewartii* is readily separated by the presence of forked, stalked, intermingled trichomes instead of exclusively simple straight ones.

3. Desideria linearis (N. Busch) Al-Shehbaz, comb. nov. Basionym: *Christolea linearis* N. Busch, in Komarov, Fl. URSS. 8: 636. 1939. *Ermania linearis* (N. Busch) Botsch., Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R. 17: 166. 1955. *Oreoblastus linearis* (N. Busch) Suslova, Bot. Zhurn. (Moscow & Leningrad) 57: 652. 1972. TYPE: Tajikistan. Pamir: Schugnan, Abchary, 2 Aug. 1904, *B. Fedtschenko s.n.* (holotype, LE!).

Ermania parkeri O. E. Schulz, Repert. Sp. Nov. Regni Veg. 31: 333. 1933. *Christolea parkeri* (O. E. Schulz) Jafri, Notes Roy. Bot. Gard. Edinburgh 22: 52. 1955. *Oreoblastus parkeri* (O. E. Schulz) Suslova, Bot. Zhurn. (Moscow & Leningrad) 57: 653. 1972. Syn. nov. TYPE: Kashmir. Sonamarg, Luderwas, 13,000 ft., 11 Aug. 1928, *R. R. Stewart 9874A* (holotype, B!).

Ermania kashmiriana Dar & Naqshi, J. Bombay Nat. Hist. Soc. 87: 274. 1990. Syn. nov. TYPE: Kashmir. Shalimar, Sonamarg (Sind Valley), 3900 m, 20 Aug. 1983, *G. H. Dar 7786* (holotype, KASH).

Ermania kachrooi Dar & Naqshi, J. Bombay Nat. Hist. Soc. 87: 277. 1990. Syn. nov. TYPE: Kashmir. Baltal, Sonamarg (Sind Valley), 3200 m, 2 Sep. 1982, *G. H. Dar 3934* (holotype, KASH; isotypes, KASH, MO!).

Plants 4–15 cm tall, densely pilose throughout to subglabrous. Trichomes simple, to 1.5 mm long. Stems simple, pilose or glabrous. Basal leaves not fleshy, pilose or glabrous, persistent; petiole 2–7 (–12) mm long, not ciliate; leaf blade broadly obovate to spatulate, 4–15 × 2–12 mm, base cuneate to attenuate, margins 3- to 5-toothed or rarely subentire, apex acute. Stem leaves similar to basal or linear to lanceolate, 5–10 × 1–3 mm, often entire, short petiolate to subsessile. Racemes 8- to 20-flowered, bracteate throughout; bracts similar to stem leaves but smaller, often adnate to pedicel. Fruiting pedicels ascending, straight, 2–8(–12) mm long, pilose or glabrous. Sepals free, oblong to ovate, 2–3 × 1–1.5 mm, caducous, pilose or with a terminal tuft of hairs, base not saccate, margins membranous. Petals purple or lavender with paler base, narrowly spatulate, 4–5(–5.5) × 1.5–2.5 mm, apex rounded; claw 2–2.5 mm long. Filaments white, slightly dilated at base, median pairs 2.5–3.5 mm long, lateral pair 1.8–2.5 mm long; anthers ovate, 0.4–0.5 mm long. Ovules 8 to 13 per locule. Fruit linear, (1.5–)2–3.5(–4.2) cm × (0.8–)1–1.7 (–2) mm, flattened; valves pilose or glabrous, distinctly veined; septum complete, membranous; style obsolete; stigma 2-lobed. Seeds brown, ovate, 0.8–1.1 × 0.5–0.8 mm, uniseriate, minutely reticulate.

Phenology. Flowering June through August. Fruiting July through September.

Habitat and distribution. Gravelly or sandy slopes, scree, gravelly moraine below glacier; 3200–5200 m. China (Xinjiang, Xizang), Kashmir, Nepal, Tajikistan.

Selected specimens examined. CHINA. **Xinjiang:** Yecheng Xian, *Li Bosheng et al. 11278* (PE); Tagdumbasch-Pamir, Pistan near Saryokol, *Alexeenko 2729* (LE). **Xizang:** without locality, *Thomson s.n.* (BM, G, GH, K, P); Ali, *Qinghai-Xizang Team 76-7948* (HNWP). INDIA. **Punjab:** Lahul, Kangra, Bara Lach La, *Koelz 6738* (GH). KASHMIR. Ishkuman Aghost, *Schmid 2449* (G). **Chitral:** Laspur (Hachin), 36°2′N, 72°27′E, *Lyon 44* (A, E); Sirogol, S of Shah Jinali Pass, *Stainton 3055* (E); Dorah Pass, Lutko valley, *Stainton 3241* (E); Amarnath, *Stainton 8709* (E); Sonamarg, Luderwas, *Stewart 9874* (B, G, MO). **Karakorum:** Gharesa Glacier base camp, 13 mi. E of Nagar, *Polunin 6133* (BM); Karakorum, Oct. 1877, *Clarke s.n.* (K). **Ladak:** above Stok, *Maxwell 92* (E); Zanskar, glacier Sentik, 34°N, 76°E, *Delouche 27* (P). **Thui region:** ca. 200 km NW of Gilgit, near watershed separating Gilgit from Chitral, *Broadhead 39* (E); Taklung La, *Koelz 6500* (GH). NEPAL. Naur Pass, *Lowndes 1159* (BM); 5 mi. S of Saldanggaon, *Polunin, Sykes & Williams 37* (BM). TAJIKISTAN. **Pamir:** N slope, river Zor-Chechekty, 12 Aug. 1948, *Stanjukovich & Kishkovsky s.n.* (LE); Chechekty, river Zor-Chechekty, *Raikova 228* (LE).

Although I have not seen the holotype of *Ermania kashmiriana*, the original description and illustration, as well as the examination of a paratype (*Dar 8301*), clearly support the placement of the species in synonymy of *Desideria linearis*. Dar and Naqshi (1990) compared *E. kashmiriana* and *E. kachrooi* with *D. stewartii* and *D. himalayensis* (all as *Ermania*), but they failed to relate their novelties to *D. linearis*. In my opinion, *E. kachrooi* is only a glabrous form of *D. linearis*, a species within a given population of which one can find glabrous and pubescent plants. In general, plants of *Desideria*, including *D. linearis*, that grow in partly shaded areas, especially under large boulders, often have the apex of the caudex elongated so that the leaf rosette appears much less congested.

Several authors (e.g., An, 1987, 1995; Kuan, 1985) followed Jafri (1955) in listing *Desideria linearis* as a synonym of *Christolea himalayensis*, but these authors erroneously recognized *C. parkeri* as a distinct species. In my opinion, the last species is only a glabrescent form of *D. linearis*. In fact, *C. parkeri* is based on *Ermania parkeri*, an invalidly published species assigned to the invalid *Ermania* (see Greuter et al. (2000) under Article 43.1).

Although Jafri (1973) maintained *Christolea parkeri*, he correctly indicated that it is not different from the earlier-published *Desideria* (as *Christolea*) *linearis*, a species that he did not recognize for Pakistan and Kashmir. However, Suslova (1972) maintained both species (as *Oreoblastus*) and separated them mainly by the presence in the former of a subapical tuft of hairs on the sepals instead of its absence in *D. linearis* and *D. himalayensis*. Obviously, this distinction is artificial, and all taxa have pubescent sepals that often are more densely hairy below the apex. The restriction of trichomes to the sepals and leaf apices is quite frequent in glabrescent forms of *D. linearis* and *D. himalayensis*.

Schulz (1931) considered *Desideria linearis* (as *Ermania parkeri*) to be closely related to *E. albiflora* (T. Anderson) O. E. Schulz, but the nearest relative of the first is *D. himalayensis*. As shown by Al-Shehbaz (2000b), *E. albiflora* belongs to the genus *Phaeonychium* O. E. Schulz.

Desideria linearis is extremely variable in the occurrence and density of the indumentum, fruit width, length, and indumentum, and number of leaf teeth. However, an examination of material from the various parts of the species range clearly negates

the need to subdivide the species into infraspecific taxa.

4. Desideria flabellata (Regel) Al-Shehbaz, comb. nov. Basionym: *Parrya flabellata* Regel, Bull. Soc. Imp. Naturalistes Moscou 43: 261. 1870. *Christolea flabellata* (Regel) N. Busch, in Komarov, Fl. URSS 8: 330. 1939. *Ermania flabellata* (Regel) O. E. Schulz, Bot. Jahrb. Syst. 66: 98. 1933. *Oreoblastus flabellatus* (Regel) Suslova, Bot. Zhurn. (Moscow & Leningrad) 57: 651. 1972. TYPE: Southern Tian Shan, Dschaman-Daban, *Sewerzow s.n.* (holotype, LE!).

Christolea pinnatifida R. F. Huang, Acta Phytotax. Sin. 35: 556. 1997. Syn. nov. TYPE: China. Qinghai: Maqen, Anyemaqen Mt., 4800 m, 25 June 1981, *R. F. Huang CG-81-154* (holotype, HNWP!).

Plants greenish, 4–15 cm tall. Trichomes simple, straight, to 1.5 mm long. Stems distinct, simple, densely pilose. Basal leaves subfleshy; petiole 2–7 mm long, pilose; leaf blade flabellate to broadly obovate, rarely spatulate, 0.6–2.5 × 0.3–2.5 cm, pilose, base cuneate to attenuate, margins (3 to)5- to 9(to 11)-toothed, rarely lowermost entire, apex acute; teeth to 10 × 3 mm. Stem leaves similar to basal. Racemes 7- to 12-flowered, ebracteate. Fruiting pedicels ascending, straight to curved, (0.5–)0.7–1.5(–2.5) cm long, spreading pilose. Sepals free, narrowly oblong, 5–8 × 1.5–2.5 mm, often persistent, pilose, base not saccate, margins membranous. Petals purple, broadly spatulate, 1.1–1.5 cm × 3.5–6 mm, apex subemarginate; claw 7–9 mm long. Filaments white to mauve, slightly dilated at base, median pairs 4.5–6 mm long, lateral pair 3–4 mm long; anthers oblong, 0.9–1.3 mm long. Ovules 7 to 12 per locule. Fruit lanceolate to lanceolate-linear, (1.7–)2.5–3.5(–4.5) cm × 2.5–5 mm, strongly flattened; valves pilose, distinctly veined; septum complete, membranous; style obsolete; stigma 2-lobed. Seeds brown, ovate, 1.3–2 × 0.9–1.2 mm, uniseriate, minutely reticulate.

Phenology. Flowering early July and August. Fruiting late July through early September.

Habitat and distribution. Alpine gravelly slopes, moraine slopes; 3300–5100 m. Afghanistan, China (Xinjiang), Kyrgyzstan, Tajikistan.

Selected specimens examined. AFGHANISTAN. Mountain above Salang tunnel, *Gibbons 823* (MO); Hindu Kush, *Gilbert 88* (E). **Parvan:** Panjshir, *Hedge & Wendelbo 5451* (E). **Kapisa:** Mir Samir area, *Gibson 211* (E). **Takhar:** Khost-o-Fereng, valley Echani-Tai, E of Chunduk, *Podlech 11832* (G). CHINA. **Xinjiang:** Kashgaria, Tian Shan, 1889, *Roborowski s.n.* (LE), *Merzbacher 354* (LE); Kashgaria, Billuli Pass, 13 June 1909, *Divnogorskaya s.n.* (LE, MO); Akto Xian, Qiaornong, *S. G. Wu, Y. H. Wu & Y. Fei 4641* (HNWP, KUN). KYRGYZSTAN. **Semirechie:** Przewalsk, river Kayche, 30 July 1913, *Shishkin s.n.* (LE), 2 Aug. 1913, *Capoznnikov s.n.* (MO). **Tianshan:** Glacier Kaïndï, *Brocherel 338* (G). TAJIKISTAN. **Shugnan:** E Bukhara, near Pass Garm-Chashmy, *Tuturin 150* (A, LE). **Pamir:** Kurushdy Glacier, *Gorbanov 191* (LE).

In every aspect of trichome morphology, flower size and color, and habit, *Christolea pinnatifida* is indistinguishable from plants of *Desideria flabellata.* The type of the former has no fruits and is rather immature. It differs only slightly from typical plants of *D. flabellata* by having slightly elongated, spatulate leaves instead of typically flabellate ones. Huang (1997a) considered *C. pinnatifida* to be related to *C. karakorumensis,* but the latter is a synonym of *D. mirabilis* and has sepals typically united instead of free. He indicated that the ovaries are glandular mamillate, but this observation was based on developing trichomes, and neither *Desideria* nor *Christolea* has any glandular trichomes or papillae.

5. Desideria incana (Ovcz.) Al-Shehbaz, comb. nov. Basionym: *Christolea incana* Ovcz., Sovetsk. Bot. 1941(1 & 2): 151. 1941. *Ermania incana* (Ovcz.) Botsch., Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R. 17: 164. 1955. *Oreoblastus incanus* (Ovcz.) Suslova, Bot. Zhurn. (Moscow & Leningrad) 57: 652. 1972. TYPE: Tajikistan. Darvaz: Mt. Masar, glacier Abdul Gassan, 11,000–12,000 ft., 23 July 1899, *V. I. Lipsky 1936* (holotype, LE!).

Plants 4–15 cm tall, densely tomentose throughout. Trichomes short-stalked forked and simple, to 1 mm long. Stems simple, tomentose. Basal leaves subfleshy, canescent, densely tomentose, persistent; petiole 0.5–2 mm long, not ciliate, unexpanded and not papery at base; leaf blade broadly obovate to spatulate, 4–13 × 2–8 mm, base cuneate to attenuate, margins 3(to 5)-toothed, sometimes subentire on sterile branches, apex acute. Stem leaves similar to basal. Racemes 6- to 20-flowered, only basally bracteate. Fruiting pedicels ascending, straight, 2–7 mm long, tomentose. Sepals free, narrowly oblong, 5–7 × 1.5–2 mm, caducous, densely tomentose, base not saccate, margins membranous. Petals purple with paler or yellowish base, spatulate, 12–18 × 4–6 mm, apex rounded; claw 7–10 mm long. Filaments white, slightly dilated at base, median pairs 5–6 mm long, lateral pair 3–4 mm long; anthers narrowly oblong, 1.2–1.5 mm long. Ovules 25 to 35 per locule. Fruit linear, 3–6.5 cm × 2.5–3.5 mm, strongly flattened; valves tomentose, distinctly veined; septum complete, membranous; style ob-

solete; stigma 2-lobed. Seeds brown, oblong, 1.2–1.5 × 0.7–1 mm, minutely reticulate.

Phenology. Flowering July. Fruiting July and August.

Habitat and distribution. Alpine gravelly areas, 3300–4600 m. Endemic to Tajikistan.

Selected specimens examined. TAJIKISTAN. **Pamir-Alay:** Sauk-Dara valley, *Ikonnikov 17878* (LE). **Bukhara:** Darvaz, range of Peter-the-Great, glacier Vereshkay, 29 July 1899, *Lipsky s.n.* (G, LE).

6. Desideria haranensis Al-Shehbaz, nom. nov. Replaced name: *Ermaniopsis pumila* H. Hara, J. Jap. Bot. 49: 200. 1974, not *Desideria pumila* (Kurz) Al-Shehbaz. TYPE: Nepal. Ca. 5 mi. SW of Saldanggaon, 26 June 1952, very loose scree, 19,500 ft., *N. Polunin, W. R. Sykes & L. H. J. Williams 24* (holotype, BM!; isotypes, A!, BM!, E!).

Plants 2–6 cm tall. Trichomes simple, straight, to 0.5 mm long, mixed on leaves with short-stalked, unequally branched forked ones. Stems erect, simple, pilose to hirsute. Basal leaves fleshy, persistent; petiole 2–12 mm long, sparsely to densely pilose with simple trichomes, ciliate at base, not expanded or papery at base; leaf blade broadly ovate, suborbicular, to obovate, 3–13 × 3–11 mm, sparsely to densely pubescent, base cuneate or obtuse, margins 1- to 5-toothed, apex obtuse. Stem leaves absent. Racemes 3- to 8-flowered, ebracteate. Pedicel divaricate, straight, 4–12 mm long, pilose. Sepals free, oblong, 3.5–4.5 × 1.7–2 mm, caducous, pilose, base not saccate, margins membranous. Petals white tinged with greenish blue, obovate, 6.5–8 × 3–4 mm, apex obtuse; claw 3–4 mm long. Filaments white, flattened, subapically toothed, median pairs 3–4 mm long, lateral pair 2–3 mm long; anthers oblong, 0.9–1.1 mm long. Ovules 5 to 7 per locule. Immature fruit linear, flattened, sessile, straight, retrorsely pilose; septum complete; style-like apex glabrous, to 1.5 mm long; stigma capitate, subentire. Seeds not seen.

Phenology. Flowering in June.

Habitat and distribution. Scree slopes; 5000–5900 m. Endemic to Nepal.

Additional specimen examined. NEPAL. Dolpo, Sya Gompa, 29°10′N, 82°59′E, *Stainton 4332* (BM, E).

Desideria haranensis is named in honor of Hiroshi Hara (5 January 1911–24 September 1986), an eminent Japanese botanist and the discoverer of this species and *D. nepalensis*. The new name is proposed because the transfer of *Ermaniopsis pumila* to *Desideria* would create a homonym of *D. pumila* (Kurz) Al-Shehbaz, which is based on the earlier published *Parrya pumila* (Kurz, 1872).

Desideria haranensis is a very rare species known thus far only from the two collections cited above. It is most closely related to *D. pumila*, from which it is distinguished by having papery instead of thickish petiolar bases, toothed instead of toothless filaments, subentire instead of 2-lobed stigmas, and several-flowered racemes instead of solitary flowers.

7. Desideria prolifera (Maxim.) Al-Shehbaz, comb. nov. Basionym: *Parrya prolifera* Maxim., Fl. Tangutica 56. 1889. *Ermania prolifera* (Maxim.) O. E. Schulz, Bot. Jahrb. Syst. 66: 98. 1933. *Christolea prolifera* (Maxim.) Ovcz., Sovetsk. Bot. 1941(1 & 2): 151. 1941. *Oreoblastus proliferus* (Maxim.) Suslova, Bot. Zhurn. (Moscow & Leningrad) 57: 652. 1972. *Christolea prolifera* (Maxim.) Jafri, Notes Roy. Bot. Gard. Edinburgh 22: 53. 1955. TYPE: China. Tibet: Kon-chun-ua, 14,500 ft., 3 July 1984, *N. M. Przewalski s.n.* (holotype, LE!; isotypes, K!, P!, PE!).

Plants scapose, villous to pilose. Trichomes simple, straight, to 1.5 mm long. Stems absent. Basal leaves subfleshy; petiole (0.2–)0.8–2(–3) cm long, persistent, sparsely to densely pilose or villous, ciliate, somewhat papery at base; leaf blade broadly ovate, suborbicular, obovate, to spatulate, 2–10 (–15) × 2–9(–12) mm, villous or pilose, base obtuse to cuneate, margins (3 to)5- to 9-toothed, rarely subentire, apex subacute. Stem leaves absent. Flowers solitary from basal rosette. Pedicel ascending-divaricate, straight, (0.2–)0.5–1.5(–2.5) cm long, villous. Sepals free, oblong, 6–7 × 2–2.5 mm, usually persistent, pilose, base not saccate, margins membranous. Petals purplish green, broadly obovate, 1.1–1.4 cm × 4–5 mm, apex subemarginate; claw 6–7 mm long. Filaments white, dilated at base, toothless, median pairs 4–6 mm long, lateral pair 3–4 mm long; anthers 1.2–1.6 mm long. Fruit linear to linear-lanceolate, (2.5–)4–6.5(–7.2) cm × (3–)4–5 mm, flattened, sessile, straight; valves obscurely veined; replum and valves pilose to villous; septum complete; style obsolete; stigma capitate, 2-lobed. Seeds oblong, 2.5–3.5 × 1.4–1.7 mm.

Phenology. Flowering July and August. Fruiting July through September.

Habitat and distribution. Scree slopes, siliceous shist; 4700–5900 m. Endemic to China (Qinghai, Xizang).

Selected specimens examined. CHINA. **Qinghai:** Bayan Har Pass, between Madoi Xian and Chindu Xian, on road between Madoi and Yushu, 34°7′N, 97°39′E, *Ho, Bartholomew, Watson & Gilbert 1684* (BM, CAS, E, HNWP, MO); Tangula Shan, Tangula Pass, 32°53′N, 91°54′E, *G. & S. Miehe 9436/08* (GOET, MO); Xue Shan Community, *Anonymous 470* (HNWP). **Xizang:** Nyainqentangula Shan, N of Damxung, 30°39′N, 91°5′E, *G. & S. Miehe, 9495/14* (GOET, MO); Mekong-Salween divide, pass E of Zongang/Wangda, 29°42′N, 98°0′E, *Dickoré 8866* (GOET, MO); Demula Shan, Basu Xian, *Qinghai-Tibet Team 73-1253* (KUN, PE); Biru Xian, Gu Teng Shan, *Qinghai-Xizang Team 11172* (KUN); Zhongba, *Qinghai-Tibet Team 6537* (KUN); peak of Sengge, near Shingkyer Yubrong, 24 July 1951, *Aufschnaiter s.n.* (BM)

Maximowicz (1889) compared *Desideria prolifera* (as *Parrya*) with *D. himalayensis* and *D. flabellata* and discussed their distinguishing characters. Both *D. prolifera* and *D. flabellata* have similar flower size and their calyces tend to persist. The principal feature separating them is that the flowers in *D. flabellata* are arranged in distinct racemes, whereas in *D. prolifera* they are solitary from the basal rosette.

8. Desideria pumila (Kurz) Al-Shehbaz, comb. nov. Basionym: *Parrya pumila* Kurz, Flora 55: 285. 1872. *Christolea pumila* (Kurz) Jafri, Fl. West Pakistan 55: 157. 1973. *Vvedenskeyella pumila* (Kurz) Botsch., Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R. 17: 176. 1955. *Solmslaubachia pumila* (Kurz) Dvořák, Folia Prirodovcd. Fak. Univ. Purkyne Brne, Biol. 13(4): 24. 1972. TYPE: Kashmir (as Tibet). Rupschu, 15,000–18,000 ft., *F. Stoliczka s.n.* (holotype, CAL?; isotype, K!).

Ermania koelzii O. E. Schulz, Repert. Sp. Nov. Regni Veg. 31: 332. 1933. TYPE: Kashmir. Rupshu, Kyensa La, 19,000 ft., 9 July 1931, *Walter Koelz 2231* (holotype, B!).

Ermania bifaria Botsch., Bot. Zhurn. (Moscow & Leningrad) 41: 730. 1956. Based on the invalidly published (see below) *E. bifaria* Botsch., Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R. 17: 164. 1955. *Oreoblastus bifarius* (Botsch.) Suslova, Bot. Zhurn. (Moscow & Leningrad) 57: 652. 1972. Syn. nov. TYPE: China. Xinjiang: Kuen-Lun, Humboldt Range, Ulan-Bulak, 4200 m, 30 June 1894, *W. Roborowski s.n.* (holotype, LE!).

Plants scapose, pilose to tomentose. Trichomes simple, straight, to 0.5 mm long, mixed on leaves with short-stalked forked ones. Stems absent. Basal leaves fleshy; petiole 2–10 mm long, persistent, densely pilose with simple trichomes, ciliate, expanded and papery at base; leaf blade broadly ovate, suborbicular, obovate, to spatulate, 2–14 × 1–11 mm, densely tomentose or pilose, base obtuse, margins 3- to 7-toothed to repand, apex obtuse. Stem leaves absent. Flowers solitary from basal rosette. Pedicel ascending-divaricate, straight, 3–10 mm long, pilose. Sepals free, oblong, 3–4 × 1.5–2 mm, caducous, pilose, base not saccate, margins membranous. Petals creamy white to purplish green, broadly obovate, 6–8 × 3–4.5 mm, apex subemarginate; claw 3–4 mm long. Filaments white, dilated at base, toothless, median pairs 3–4 mm long, lateral pair 2–2.5 mm long; anthers narrowly oblong, 0.9–1.2 mm long. Ovules ca. 7 per locule. Immature fruit oblong-linear to linear-lanceolate, 1–2 cm × 2–3 mm, flattened, sessile, straight, retrorsely pilose along replum; valves glabrous; septum complete; style obsolete; stigma capitate, 2-lobed. Seeds not seen.

Phenology. Flowering June and July.

Habitat and distribution. Limestone, mica shist; 4200–5800 m. China (Xinjiang,? Xizang), Kashmir.

Botschantsev's (1955) description of *Ermania bifaria* was invalid because he placed the species in what was then an invalidly published genus. When he (Botschantsev, 1956) validated *Ermania*, he listed *E. bifaria* with full reference to his earlier work. Therefore, the correct date of the valid publication of *E. bifaria* should be Botschantsev's 1956 instead of 1955 work.

Botschantsev (1955) recognized two species in *Vvedenskeyella* Botsch., of which the generic type, *V. kashgarica* Botsch., has been transferred to *Phaeonychium* (Al-Shehbaz, 2000b). The second species, which is based on *Parrya pumila*, is assigned here to *Desideria*. Apparently, Botschantsev did not examine the type material of *P. pumila*, as evidenced from his description of the same species as *Ermania bifaria*. *Phaeonychium* differs from *Desideria* by having a thick and compact instead of slender and rhizome-like caudex, pinnately veined instead of often palmately veined leaves, incumbent instead of accumbent cotyledons, and fruit valves without instead of with prominent marginal veins.

Desideria pumila was said to occur in Xizang (Kuan, 1985; An, 1987), but I have not seen any material other than the types cited above, which were collected from Rupshu, Kashmir. Jafri (1973) considered the species (as *Christolea*) to be very closely related if indeed different from what he called *C. lanuginosa* (Hook. f. & Thomson) Ovcz. However, the last species is clearly unrelated to *D. pumila* and has been treated in *Eurycarpus* by Al-Shehbaz and Yang (2000a).

9. Desideria baiogoinensis (K. C. Kuan & Z. X. An) Al-Shehbaz, comb. nov. Basionym: *Christolea baiogoinensis* K. C. Kuan & Z. X. An, in C. Y. Wu, Fl. Xizang. 2: 388. 1985. TYPE: China. Xizang: Baiogoin, 5100 m, 18 June 1976, *K. Y. Lang 9460* (holotype, PE!; isotype, PE!).

Plants scapose, villous. Trichomes simple and short-stalked forked, straight, to 1 mm long. Stems absent. Basal leaves subfleshy; petiole 0.4–1.6 cm long, persistent, villous, ciliate, somewhat papery at base; leaf blade broadly ovate, suborbicular, or obovate, 4–8 × 3–6 mm, villous, base obtuse to cuneate, margins 3- to 7-toothed, apex acute. Stem leaves absent. Flowers solitary from basal rosette. Pedicel ascending-divaricate, straight, 0.5–2 cm long, villous. Sepals free, oblong, 4–6 × 1.5–2.5 mm, usually persistent, pilose, base not saccate, margins membranous. Petals purplish, broadly obovate, 7–1.2 cm × 3.5–4.5 mm, apex subemarginate; claw 4–6 mm long. Filaments white, dilated at base, toothless, median pairs 3.5–5 mm long, lateral pair 2–2.5 mm long; anthers 1–1.2 mm long. Ovules 15 to 20 per locule. Fruit ovate to lanceolate, 1–2.5 cm × 6–9 mm, flattened, sessile, straight; valves prominently reticulate veined; replum and valves villous; septum complete; style 0.5–1 mm long; stigma capitate, 2-lobed. Seeds oblong, 1.5–2 × 0.8–1.1 mm.

Phenology. Flowering June and July. Fruiting July and August.

Habitat and distribution. Open sand and gravel; 4700–5600 m. Endemic to China (Qinghai, Xizang).

Selected specimens examined. CHINA. **Xizang:** Amdo Xian, *Tao Deding 10819* (HNWP, KUN); Baingoin Xian, *Lang Kaiyong 9469* (PE), *9487* (KUN); Tumain, *Yang Jinxiang 1884* (KUN), *Qinghai-Xizang Team 177* (HNWP); NE plateau along Golmud–Lhasa hwy, Wudaoliang-Tanggulashanqu, 34°35′N, 92°44′E, *G. & S. Miehe 9416/00* (GOET, MO).

The species was included in the *Flora of Qinghai* (Huang, 1997b), but I have not seen any material from that province.

10. Desideria mirabilis Pamp., Bull. Soc. Bot. Ital. 1926: 111. 1926. *Christolea mirabilis* (Pamp.) Jafri, Fl. West Pakistan 55: 160. 1973. TYPE: [Kashmir.] Karakorum; above Caracash Valley, Chisil Gilgha Pass, 5360 m, 28 June 1914, *G. Dainelli & O. Marinelli 2* (lectotype, here designated, FI, photo!).

Christolea scaposa Jafri, Notes Roy. Bot. Gard. Edinburgh 22: 58. 1955. Syn. nov. TYPE: Kashmir. Shaksgam Valley, 4950 m, 3 July 1926, *R. C. Clifford 7* (holotype, K!).

Christolea karakorumensis Y. H. Wu & Z. X. An, Acta Phytotax. Sin. 32: 577. 1994. Syn. nov. TYPE: China. Xinjiang: Pishan (Guma), Shenxianwan, 5250 m, 25 July 1989, *Karakorum-Kunlun Expedition 5100* (holotype, HNWP!, listed as NWBI).

Desideria pamirica Suslova, Novosti Sist. Vyssh. Rast. 10: 163. 1973. Syn. nov. TYPE: Tajikistan. Pamir: above Czeczekty, near Zor, 4900 m, 10 Aug. 1970, *T. Suslova s.n.* (holotype, LE!).

Christolea suslovaeana Jafri, Fl. West Pakistan 55: 158. 1973, not *Christolea pamirica* Korshinsky, Mém. Acad. Imp. Sci. Saint Pétersbourg, ser. 8, 4: 89. 1896. TYPE: same as that of *Desideria pamirica*.

Plants 2–10 cm tall. Trichomes simple and to 1.5 mm long, rarely mixed with forked ones. Stems distinct, simple, densely pilose. Basal leaves subfleshy; petiole 3–15 mm long, densely pilose, not expanded or papery at base; leaf blade flabellate to spatulate-orbicular, (2–)5–15 × 3–9(–15) mm, pilose, base cuneate, margins 8- to 10-toothed, apex acute; teeth to 8 mm long. Stem leaves similar to basal. Racemes 8- to 20-flowered, ebracteate. Fruiting pedicels ascending, straight to curved, 5–10 mm long, spreading pilose. Sepals united, (2.5–)3.5–5.5 × 1.5–2.5(–3) mm, persistent, densely pilose, base not saccate; calyx lobes ovate, unequal, 0.5–2 mm long, margins membranous. Petals purple to purplish green with yellowish base, obovate, 5–8 × (1.5–)2.5–3 mm, apex obtuse; claw 2.5–4 mm long. Filaments white, slightly dilated at base, median pairs (3–)4–5 mm long, lateral pair (2–)2.5–3.5 mm long; anthers oblong, 0.5–0.8 mm long. Ovules 12 to 18 per ovary. Fruit linear, 1–2(–3) cm × ca. 2 mm, slightly flattened to subterete; valves pilose, distinctly veined; septum perforate or reduced to a narrow rim, membranous; style obsolete to 0.7 mm long; stigma 2-lobed. Seeds oblong, 1.5–1.8 × 0.8–1 mm, papillate.

Phenology. Flowering July and August. Fruiting August and early September.

Habitat and distribution. Gravelly slopes; 4000–5000 m. China (Xinjiang), Kashmir, Tajikistan.

Selected specimens examined. KASHMIR. **Karakorum:** above Caracash Valley, Chisil Gilgha Pass, 5300 m, 21 June 1914, *Dainelli & Marinelli 2* (photo, FI). TAJIKISTAN. **Pamir:** Badakhshan Mt., close to river Maldzhuran, *Tzvelev 700* (LE); Badakhshan Mt., 7 km N of Pass Takhta-Korum, *Tzvelev 1060a* (LE); Badakhshan Mt., basin of river Pshart, *Tzvelev 535* (LE); valley of river Chunjabay, *Kuzmina 6060* (LE); Checkekty slope, 19 Aug. 1965, *Ikonnikov s.n.* (LE).

Although Pampanini (1926) did not cite any collections within the original description of *Desideria mirabilis*, he listed in the preceding discussion

three localities from which the species was collected, and he (Pampanini, 1930) gave the details of these three syntypes.

By their reduction of *Desideria mirabilis* to synonymy of *Cheiranthus himalayensis* (as *Ermania* or *Christolea*), Schulz (1927a, 1936), Botschantsev (1955, 1956), and Jafri (1955) overlooked the fact that the latter species has bracteate instead of ebracteate racemes, septate instead of eseptate fruits, free instead of united sepals, and fruits 3–4.5 mm instead of ca. 2 mm wide. The differences between the two species are so significant that it is hard to imagine they are conspecific.

Although they correctly noticed that the sepals in *Christolea karakorumensis* are united, Wu and An (1994) were probably unaware of *Desideria mirabilis*, a species endemic to the Karakorum Mountains and indistinguishable in every aspect from their novelty.

Jafri (1955) did not mention the connation of sepals in his original description of *Christolea scaposa*, though the illustration clearly shows gamosepalous calyces. By contrast, his (Jafri, 1973) illustration did not show gamosepaly accurately, though both illustrations were based on the same specimen. However, he mentioned that the sepals are "rarely connate below." Jafri (1955) suggested that *C. scaposa* is related to *C. prolifera*, while he (Jafri, 1973: 158) indicated that the species is closely related to *Desideria suslovaeana* except for "the absence of septum and slight difference in leaves." In my opinion, *C. scaposa* is indistinguishable from *Desideria mirabilis* and is unrelated to *D. prolifera*. The latter has solitary flowers and deciduous polysepalous calyces, whereas *D. mirabilis* has the flowers in racemes and persistent gamosepalous calyces.

11. Desideria nepalensis H. Hara, J. Jap. Bot. 50: 264. 1975. TYPE: Nepal. Barum Valley, 17,700 ft., 26 May 1954, *L. W. Swan 71-72* (holotype, BM!).

Plants 2–3 cm tall. Trichomes simple, straight, to 1 mm long. Stems minute, simple, glabrous. Basal leaves subfleshy; petiole 2–5 mm long, sparsely pilose with simple trichomes, ciliate at base, not expanded or papery at base; leaf blade broadly obovate to subflabellate, 2–3 × 1–3 mm, densely pubescent, base cuneate, margins 3- to 5-toothed, apex acute. Stem leaves absent. Flowers 2–4, ebracteate. Pedicel ascending, straight, 3–5 mm long, solitary from basal rosette, spreading pilose. Sepals united, 5–6 × 3–4 mm, densely pilose, base not saccate; calyx lobes ovate, 1.5–2 mm long, margins membranous. Petals ?purplish, obovate, 11–13 × 5–6 mm, apex obtuse; claw 6–7 mm long. Filaments slightly dilated at base, median pairs 4.5–5.5 mm long, lateral pair 3–4 mm long; anthers oblong, 0.9–1.1 mm long. Ovule number, fruits, and seeds unknown.

Desideria nepalensis is known only from the type collection made at an altitude of about 5400 m. It is readily distinguished from *D. mirabilis* by its much larger flowers (see key).

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ADAPTIVE RADIATION OF POLLINATION MECHANISMS IN *IXIA* (IRIDACEAE: CROCOIDEAE)[1]

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ABSTRACT

Field observations, floral dissections, and pollen load analyses of insects captured on 20 species of *Ixia* (Iridaceae), representing examples of the four major floral types in the genus, indicate that this southern African genus of 52 species is cross pollinated by a wide variety of insects. The pollination ecology of *Ixia* species can be divided into several distinct systems exploiting insects of four insect orders (Coleoptera, Diptera, Hymenoptera, and Lepidoptera). *Ixia atrandra, I. curta, I. lutea, I. maculata, I. metelerkampiae,* and *I. versicolor* have salver-shaped, nectarless flowers, in bright colors contrasting with dark "beetle marks" and are pollinated exclusively by hopliine scarab beetles. Four *Ixia* species with narrowly tubular flowers, spreading tepals, and ample nectar are pollinated by long-proboscid flies (*Moegistorhynchus longirostris* and *Philoliche* species). Three additional species with tubular flowers, and modest nectar volumes, appear to be pollinated by the pieriid butterfly, *Colias electo* (*Ixia orientalis*), or by a combination of hopliine beetles and tabanid flies with short probosces (*I. aurea, I. esterhuyseniae, I. tenuifolia*). The remaining species are largely pollinated by anthophorine bees or *Apis mellifera,* but bee pollination comprises three discrete systems. Species pollinated by *Anthophora* and *Pachymelus* species (*I. capillaris, I. latifolia, I. odorata, I. rapunculoides, I. thomasiae*) have cup-shaped flowers that secrete nectar. Salver-shaped flowers of *I. flexuosa* secrete no nectar, but are pollinated by pollen-collecting *Apis mellifera.* Buzz pollination by *Amegilla fallax* in *I. scillaris* is associated with vertical floral presentation, nectarless flowers, unusual in having a short, closed perianth tube, short, stubby filaments, and anthers dehiscing incompletely from the base. Outgroup comparison suggests that the ancestral pollination system in *Ixia* is the one in which flowers are cup-shaped, produce nectar, and are pollinated by large anthophorine bees. Exaggeration of the perianth into an elongate tube containing ample nectar, or the closure of the perianth tube and absence of nectar, or the development of basal anther dehiscense must be regarded as specialized adaptations related to their derived pollination strategies.

Key words: Apidae, Hopliini, Iridaceae, *Ixia,* Nemestrinidae, pollination biology, Tabanidae.

Macroevolution of the African Iridaceae depends in part on the plasticity of pollination mechanisms. *Nivenia* (10 spp.) appears to be one of the few genera of any size in which species are pollinated primarily by two pollinator groups, long-proboscid flies (Nemestrinidae) or long-tongued anthophorine bees (Goldblatt & Bernhardt, 1990). In contrast, the larger genera of African Iridaceae are characterized by a diversity of pollination systems. For example, *Lapeirousia* consists of species pollinated by long-proboscid flies, bees and butterflies combined, or moths (Goldblatt et al., 1995). *Romulea* species exploit scarab beetles, pollen-collecting bees or, in one case, nemestrinid flies (Goldblatt et al., 1998a; Manning & Goldblatt, 1996, and unpublished). The majority of species of *Gladiolus* appear to be pollinated primarily by nectar-feeding anthophorine bees (Goldblatt et al., 1998b), but recent fieldwork also indicates that some red-flowered species are pollinated by the large butterfly *Aeropetes* (Johnson & Bond, 1994), while other species are dependent on andrenid bees, a combination of these bees and hopliine beetles (Goldblatt et al., 1998a), long-proboscid flies, moths, or birds (Goldblatt & Manning, 1998). The adaptive radiation of floral characters thus appears to have played a prominent role in evolution and speciation within African Iridaceae.

Ixia, a moderate-sized genus of Iridaceae subfamily Crocoideae Burnett, Outl. Bot.: 451 Jun. 1835. (syn. Ixioideae Klatt, 1866 as subordo Ixieae), comprises some 52 species (Lewis, 1962; de Vos, 1999; Goldblatt & Manning, 1999, 2000). The genus extends from Namaqualand in the northwest of the subcontinent to Eastern Cape Province near Grahamstown in the east, a range that coincides almost exactly with the southern African winter-rainfall zone. Species diversity is highest in the

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geographical center of this zone, the Western Cape Province. At first glance, *Ixia* appears to exhibit only moderate floral diversity compared, for example, with the ca. 245 sub-Saharan African species of *Gladiolus*, the 52 species of *Watsonia*, or the ca. 40 species of *Lapeirousia*. All *Ixia* species have radially symmetric perianths and relatively small flowers 1.5–3 cm in diameter, typically arranged in a dense spike at the apex of a slender flowering stem. The androecium is also symmetric in the majority of species, and the style diverges into three branches near the mouth of the floral tube, at or below the level of the anthers. On the other hand, *Ixia* flowers vary remarkably in the range of perianth color and pigmentation patterning, perianth tube length and structure, staminal coalescence and mode of anther dehiscence, and nectar production. A particularly unusual aspect of floral variation within this genus is that while the anthers of most Iridaceae are longitudinally dehiscent and mounted on long filaments, species of *Ixia* subg. *Dichone* have short, stubby filaments and short, inflated anthers with a narrow connective. The anthers of all but one of the nine species of this subgenus dehisce incompletely, and are more or less basally porose in some species (Lewis, 1962).

Field study of the pollination systems of a range of *Ixia* species was undertaken to obtain additional information about the evolution of adaptive mechanisms, to provide insights into the way the flowers of *Ixia* species function, and to help understand the seeming contradiction in apparently simple and not particularly diverse floral morphology and a range of quite distinct pollination systems.

Methods

Floral phenology, life span, and floral presentation

Direct observations are presented on 20 *Ixia* species made during the years 1993 to 1999 in the field and in living collections at Kirstenbosch Botanic Gardens, Cape Town. Observations on the pollination biology of *Ixia* were made in the course of other field research in the southern spring at various sites (Table 1) in the southwestern Cape and the western Karoo, South Africa, areas of Mediterranean climate with wet winters and dry summers. Observations of insect foraging involved 4–10 hours per plant species and included recording of both floral attractants (pigment patterns, scent), the mode and timing of anthesis (opening of individual buds), anther dehiscence, expansion of stigmatic lobes, the behavior of insects on the flower, and the taxonomic diversity of floral foragers. The range of species studied includes examples from all infrageneric taxa (de Vos, 1999) and all the major floral types except *I. acaulis*, a rare limestone endemic, which has long-tubed yellow flowers borne at ground level and a subterranean ovary (Goldblatt & Manning, 1993).

Floral scent was noted in the field and in cultivated plants. Scents too weak to be discerned by the human nose were recorded after individual flowers were picked and placed in clean, lidded glass jars and stored in a warm place. The contents of each jar were smelled after a minimum of 60 minutes (Buchmann, 1983).

Nectar analysis

Nectar volume measurements were taken primarily from unbagged flowers in the field, reflecting both rates of secretion and depletion, but some species were sampled in the laboratory where no insects visited the flowers. Nectar sugar chemistry and concentration are unlikely to be affected significantly, as opposed to sampling bagged flowers. Ideally both bagged and unbagged flowers should be sampled. Studies on nectar characteristics of *Lapeirousia* (another southern African genus of Iridaceae–Crocoideae) suggest that nectar concentration is not affected using unbagged flowers versus those examined in the laboratory where insects were excluded (Goldblatt et al., 1995). Nectar volume is expected to be lower in unbagged versus bagged flowers, but sampling of nectar of unbagged flowers in populations being visited by pollinators reflects a realistic situation that confronts a particular pollinator in the field. To collect nectar whole flowers were picked and nectar was withdrawn from the base of the perianth tube with 3 μl capillary tubes after separating the ovary from the perianth base. The percentage of sucrose equivalents in fresh nectar was measured in the field or laboratory on a Bellingham and Stanley hand-held refractometer (0–50%) from five or more individuals per population, unless fewer individuals were available. Additional nectar samples were dried on Whatmans filter paper no. 1 and sent to B.-E. van Wyk, Rand Afrikaans University, Johannesburg, for HPLC nectar chemistry analysis.

Insect observation, pollination mechanisms, and pollen load analysis

Observations of insects on *Ixia* flowers included whether insects contacted anthers and stigmas while foraging. Insects observed probing the floral tube or brushing the anthers or stigmas were captured and killed in a jar using ethyl acetate fumes.

Table 1. Study sites and voucher information for species studied. Vouchers are housed at MO (Goldblatt) or at NBG (other collectors). All study sites are in South Africa.

Species	Study site	Voucher
Subgenus *Ixia*		
Section *Morphixia*		
I. aurea Goldblatt & J. C. Manning	Western Cape, near Darling	*Goldblatt & Manning 10338*
I. capillaris L. f.	Western Cape, near Bot River	*Goldblatt 10674*
I. esterhuyseniae M. P. de Vos	Western Cape, Landrostkop	*Oliver & Oliver 11461*
I. latifolia D. Delaroche (site 1)	Western Cape, Cold Bokkeveld	*Thompson 1550*
(site 2)	Western Cape, near Touwsrivier	*Goldblatt & Nänni 11202*
I. odorata Ker Gawl.	Western Cape, Darling, Waylands	*Burgers 1229*
I. orientalis L. Bolus	Western Cape, near Caledon	*Goldblatt & Manning 9789*
I. rapunculoides Delile	Northern Cape, Bokkeveld Plateau	*Pretorius 75*
I. tenuifolia Vent.	Western Cape, Camphill road	*Goldblatt & Manning 10333*
I. thomasiae Goldblatt	Western Cape, near Middelpos	*Goldblatt & Manning 10368*
Section *Hyalis*		
I. longituba N. E. Br.	Western Cape, near Greyton	*Goldblatt & Manning 10341*
I. paniculata D. Delaroche (site 1)	Western Cape, Rondeberg	*Goldblatt & Manning 10051*
(site 2)	Northern Cape, Nieuwoudtville Waterfall	*Goldblatt & Manning 10429*
I. paucifolia G. J. Lewis (site 1)	Western Cape, Burger's Pass	*Goldblatt & Manning 10012*
(site 2)	Western Cape, Worcester district, near De Wet	*Goldblatt & Manning 9690*
Section *Ixia*		
I. atrandra Goldblatt & J. C. Manning	Western Cape, Brandvlei Hills	*Goldblatt 10568*
I. curta Andrews	Western Cape, Versveld Reserve, Darling	*Goldblatt & Manning 10358*
I. flexuosa L.	Western Cape, Bot River	*Goldblatt 10671*
I. lutea Eckl. (site 1)	Western Cape, Darling Nature Reserve	*Goldblatt 11151*
(site 2)	Western Cape, Elandsberg Farm	*Goldblatt* no voucher
(site 3)	Western Cape, Piketberg	*Goldblatt & Manning* no voucher
I. maculata L. (site 1)	Western Cape, Waylands, Darling	*Goldblatt & Manning 10386A*
(site 2)	Western Cape, Clanwilliam, Farm Ysterfontein	*Goldblatt & Manning 10349*
(site 3)	Western Cape, near Leipoldtville	*Goldblatt & Manning 10324*
I. metelerkampiae L. Bolus	Western Cape, Bainskloof	*Lewis 5678*
I. versicolor G. J. Lewis	Western Cape, Strand	*Goldblatt & Manning 11031*
Subgenus *Dichone*		
I. scillaris L. (site 1)	Western Cape, Lion's Head, above Camps Bay, Cape Town	*Barker 2214*
(site 2)	Western Cape, Darling Nature Reserve	*Goldblatt 1196A*

Pollen was removed from insects after specimens were pinned. To prevent contamination of the body of an insect with pollen carried by another in the same jar, each insect was wrapped in tissue as soon as it was immobilized by jar fumes. Body length and proboscis length of insects were recorded from captured specimens. Capturing a bee or fly at any site appeared to reduce the insect population significantly. We therefore killed as few insects as necessary to obtain specimens for identification and pollen load analysis. Removal of pollen from insect bodies involved gently scraping pollen off the body, including the scopae or corbiculae of bees, with a dissecting needle (see Goldblatt et al., 1998a, b). The residue from needle probes was collected on glass slides and mounted in 1–2 drops of Calberla's fluid (Ogden et al., 1974). In the case of flies, which are comparatively large insects, sites of pollen deposition are quite discrete for a particular plant visited, and pollen species can usually be identified without recourse to microscopic examination. Pollen grains were identified microscopically by com-

parison with a reference set of pollen grain preparations made from plants flowering at the study sites. *Ixia* pollen grains are recognized by their large size, perforate-scabrate exine, and monosulcate aperture with prominent 1-banded operculum, the latter feature unique as far as is known (Goldblatt et al., 1991).

Insect specimens were identified by R. W. Brooks (Apidae), University of Kansas, H. Dombrow, Worms, Germany (Scarabaeidae), and J. C. Manning (Diptera, Lepidoptera). Voucher specimens are deposited at the Natal Museum, Pietermaritzburg, South Africa, or the Snow Entomological Museum, Lawrence, Kansas.

Results

Floral phenology, life span, floral presentation, and attractants

Floral phenology and life span. Species of *Ixia* are seasonal, corm-bearing geophytes of small to moderate size, typically 15–40 cm high (Fig. 1). Individuals produce a single, simple or branched flowering stem annually, and flowering is closely synchronized in a population. Inflorescences are spikes with helically arranged flowers (Lewis, 1962). With a few exceptions, flowering occurs in late winter and spring (August to October) (Table 2). This coincides with the period of optimal plant growth, during or soon after the main rainy season.

The pattern of flower buds opening on an inflorescence is acropetal. In all species a mature bud expands in the early to late morning, and the open flower typically lasts three or four days. Flowers open sequentially, usually one day apart, hence there are often three or four flowers open at any time on an inflorescence. At sunset the tepals loosely close to enfold the anthers and stigmas. Tepals unfold again the next day, between 08.00 and 12.00 hours, depending on species and to some extent on ambient temperature. On cold (< 15°C) or overcast days they may unfold partially or not at all.

Flowers of all species studied show weak mechanical protandry. In most species the anthers dehisce longitudinally within one to four hours after the tepals first unfold (Fig. 2A, B, C). In these species pollen remains in place and exposed in the anthers. In contrast, in most species of section *Di-*

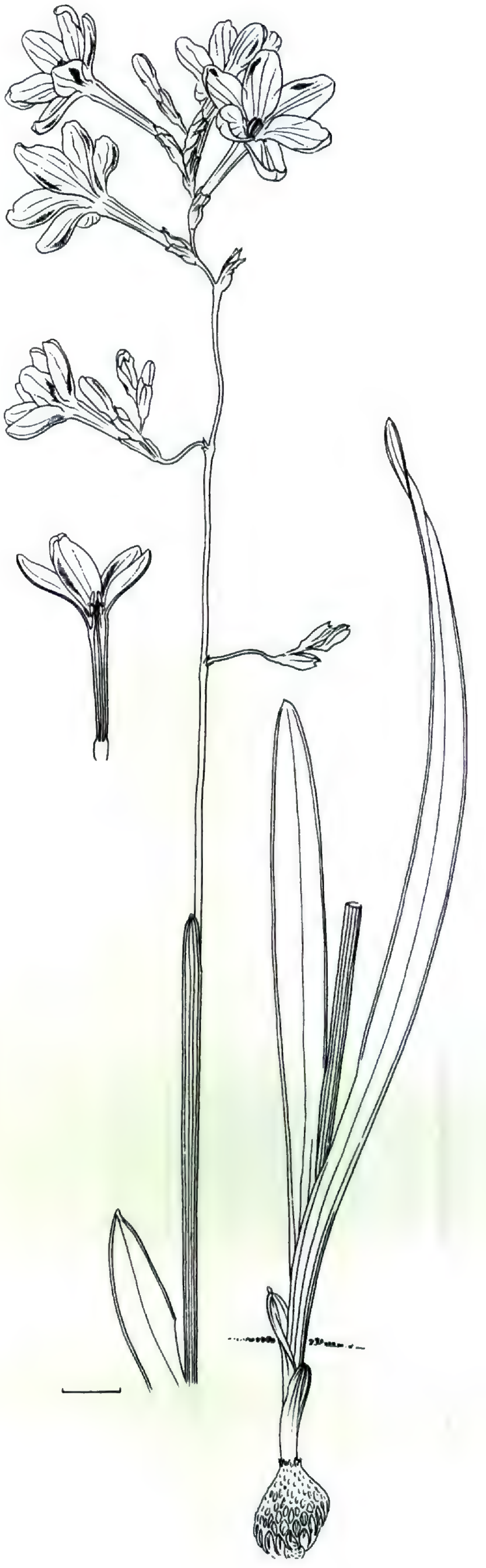

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Figure 1. *Ixia paucifolia*, a typical member of the genus, showing general habit, inflorescence, and flowers (scale bar 1 cm).

Table 2. Floral characteristics of *Ixia* species arranged according to flower type. + = presence, − = absence, tr = trace amount too little to measure volumetrically. Conventional perianth tubes are cylindrical and hollow (indicated by +), but in closed tubes the perianth tissue tightly envelops the style so that the interior of the tube is filled by stylar tissue (indicated by −); additionally, in these species the filaments are contiguous and sometimes fused, thus closing the mouth of the tube.

Species	Flower		Perianth tube		Beetle marks	Nectar presence	Flowering time
	shape	color	length mm	open			
Subgenus *Ixia*: section *Morphixia*							
I. aurea	salver	orange	ca. 8	+	−	+	Sep.–Oct.
I. capillaris	cup	blue	ca. 7	+	−	+	Aug.–Sep.
I. esterhuyseniae	cup	yellow	4–5	+	−	+	Nov.
I. latifolia	cup	pink to mauve	12–14	+	−	+	Aug.–Sep.
I. odorata	cup	yellow	ca. 8	+	−	+	Sep.
I. orientalis	salver	mauve	ca. 8	+	−	+	Oct.
I. rapunculoides	cup	blue-mauve	ca. 10	+	−	+	Aug.–Sep.
I. tenuifolia	salver	orange	ca. 12	+	+	tr	Sep.
I. thomasiae	cup	pink	ca. 7	+	−	+	Oct.
Section *Hyalis*							
I. longituba	tube	pink	30–32	+	−	+	Oct.
I. paniculata	tube	cream	65–75	+	−	+	Oct.–Nov.
I. paucifolia	tube	cream	16–28	+	−	+	Aug.–Sep.
Section *Ixia*							
I. atrandra	salver	cream	ca. 10	−	+	−	Oct.–Nov.
I. curta	salver	orange	ca. 12	−	+	−	Sep.–Oct.
I. flexuosa	salver	white/pink	ca. 6	−	−	−	Aug.–Sep.
I. lutea	salver	cream	6–12	−	+	−	Aug.–Sep.
I. maculata	salver	orange	ca. 8	−	+	−	Sep.–Oct.
I. metelerkampiae	salver	mauve	ca. 4	−	+	−	Oct.–Nov.
I. versicolor	salver	white/pink	ca. 10	−	+	−	Sep.–Oct.
Subgenus *Dichone*							
I. scillaris	salver	pink	3–4	−	−	−	Oct.

chone the anther dehiscence is virtually porose, starting from the base (Fig. 2D). Pollen in these species is not shed but remains within the anthers unless actively removed by an insect. The time of anther dehiscence depends to some extent on ambient temperature and humidity, and anthers dehisce later in wet cool conditions. The three stylar lobes, the distal adaxial surfaces of which comprise the stigmas, are held together when the flower first opens, but they diverge later during the same day.

We did not determine pollen-stigma compatibility ourselves, but Horn (1962) showed that a range of species are self-compatible, including *Ixia maculata* and *I. polystachya,* which were studied here, but have reduced fruit and seed production when selfed by hand compared with xenogamous crosses. Horn also reported that *I. odorata* was self-incompatible. Mechanical selfing is restricted in *Ixia scillaris* by the spatial separation of anthers and stigmatic surfaces.

Floral presentation and attractants. Open flowers are typically held erect to suberect in a fairly congested spike (Fig. 1). *Ixia viridiflora* (sect. *Morphixia*), not studied here, has elongate, lax spikes, while species of subgenus *Dichone* have comparatively sturdy stems and flowers held horizontally and relatively widely spaced on the spike axis. *Ixia odorata* is one of two species examined that produces an odor, and the flowers have a sweet scent reminiscent of commercial cultivars of *Viola odorata.* In the other scented species, *I. flexuosa,* the flowers have a sour, acrid, musky odor, unpleasant to the human nose.

Floral presentation (including shape, pigmentation, attractants, and rewards) in *Ixia* falls into four main categories (Table 2), although the basic perianth plan is always symmetrical and actinomorphic. The first category, of which *I. latifolia* and *I. rapunculoides* (sect. *Morphixia*) are typical examples, have cup- or salver-shaped flowers (Fig. 3). The floral tube is funnel-shaped with a hollow, narrow cylindric basal half and a flared upper half, this

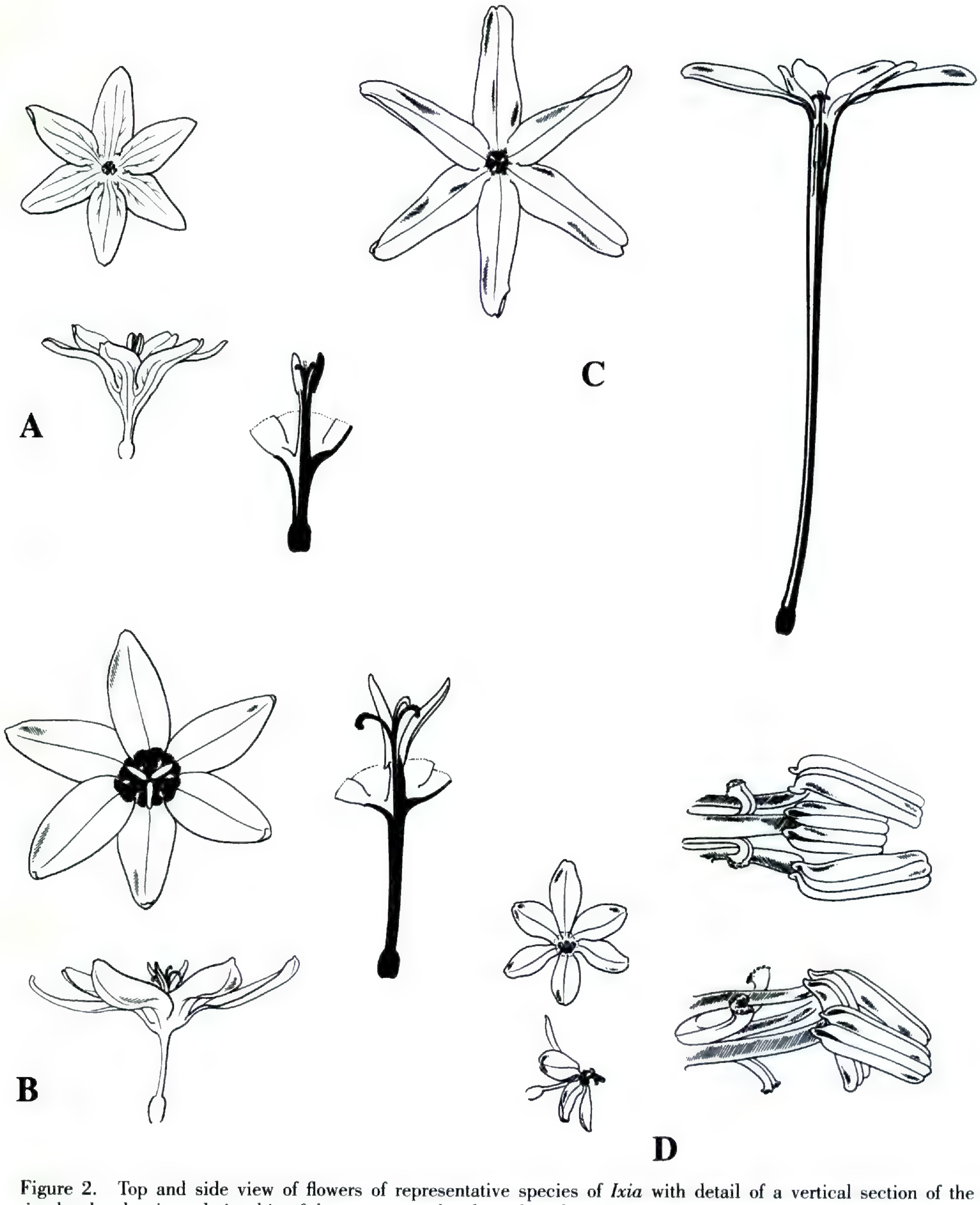
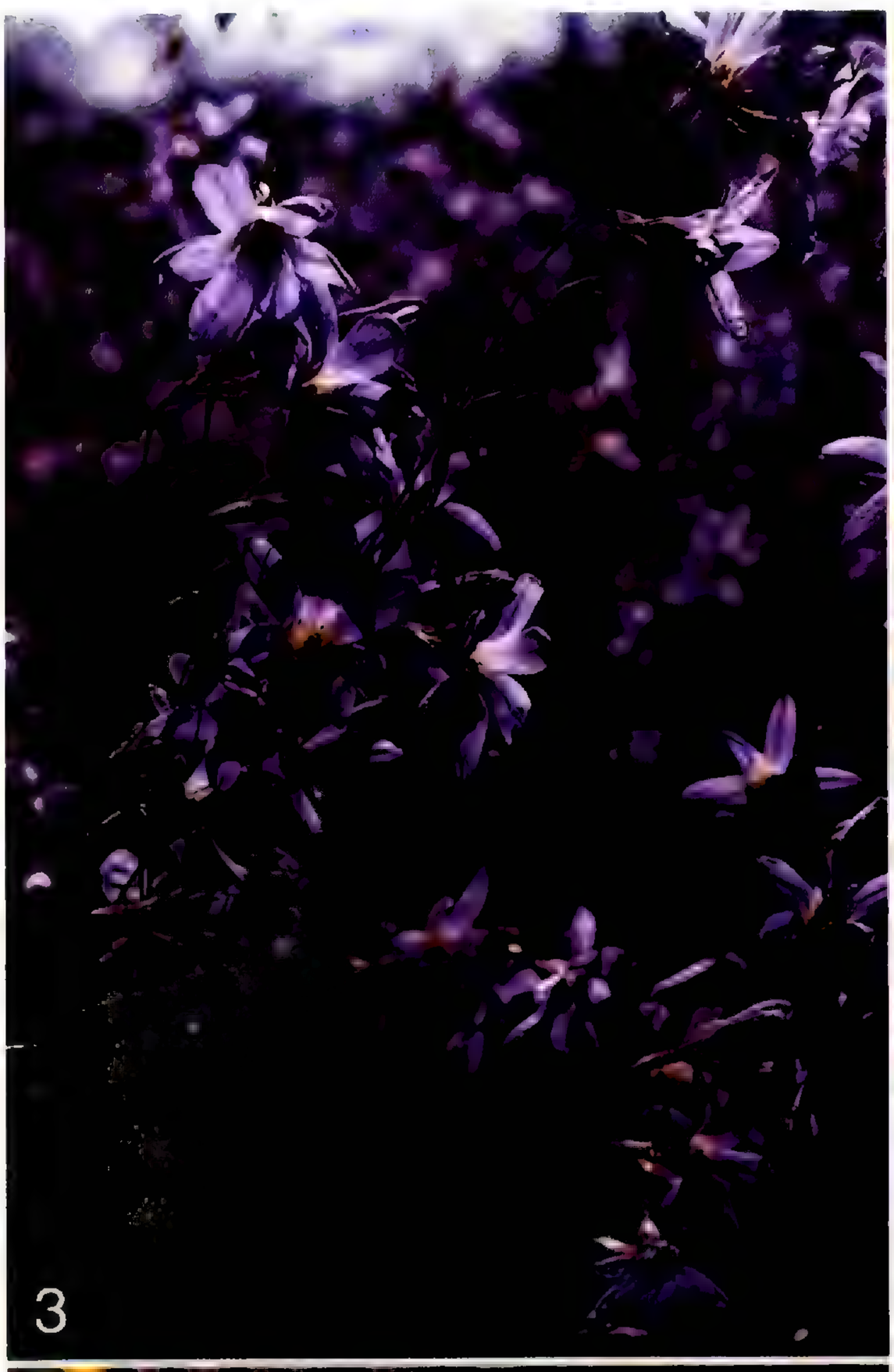


Figure 2. Top and side view of flowers of representative species of *Ixia* with detail of a vertical section of the perianth tube showing relationship of the stamens and style to the tube. —A. *Ixia rapunculoides* (sect. *Morphixia*), with a wide floral tube and longitudinally dehiscent anthers. —B. *I. maculata* (sect. *Ixia*), with a narrow floral tube tightly enclosing the style and prominent, longitudinally dehiscent anthers. —C. *I. paniculata* (sect. *Hyalis*), with elongate, hollow tube with included stamens. —D. *I. scillaris* (sect. *Dichone*), with unilateral anthers dehiscing from the base. Whole flowers full size, details variously enlarged (drawn by John Manning).

ranging from fairly wide and 7–10 mm wide at the mouth or narrow and only 2–4 mm wide at the mouth. The cylindrical part of the tube contains nectar, which may extend upward into the base of the flared portion. The stamens are symmetrical and located around the center of the flower and either within the wide part of the floral tube or are shortly to fully exserted. Anthers are extrorse with loculicidal dehiscence, and the pollen adheres to the dehisced anther locules (Lewis, 1962). The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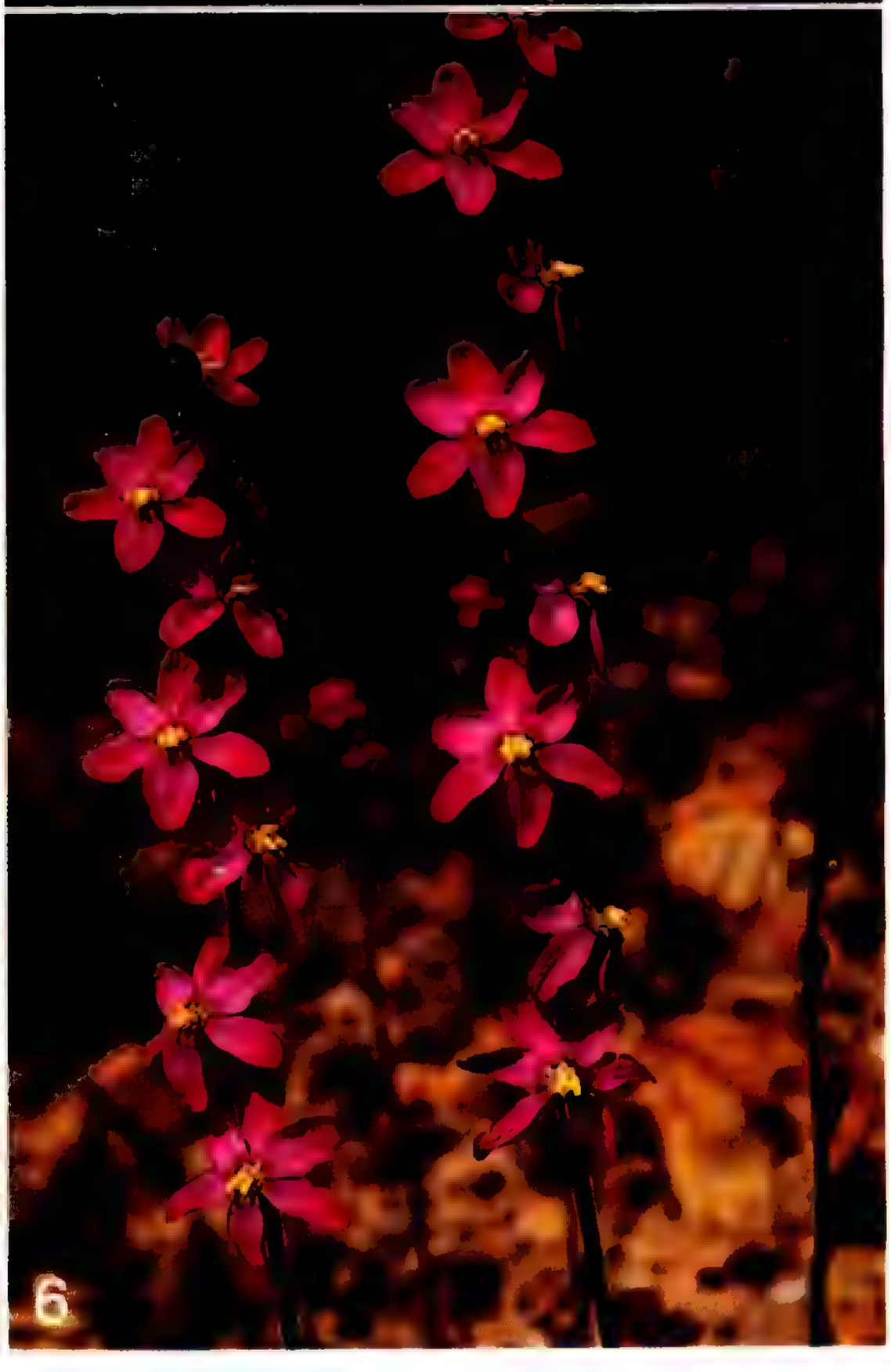
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style is central in all species and divides apically into three lobes that extend between the filaments or the lower half of the anthers, depending on the level at which the style divides. Perianth coloration in flowers of this group is usually pink to mauve or blue but may sometimes be deep pink or magenta in forms of *I. latifolia*. The fragrant flowers of *I. odorata* are yellow and unusually small and crowded together, collectively forming the prominent signal to pollinators. *Ixia aurea* and *I. tenuifolia* are exceptional among the species with tubular flowers in having bright yellow to orange perianths, marked in the latter with a dark reddish center.

A second flower category is exemplified by *Ixia paucifolia* (Fig. 1) and *I. paniculata* (Figs. 2C, 4) and is found in all species of section *Hyalis* (Table 2). The floral tube is hollow, cylindric, much exceeds the tepals, and has a narrow diameter ca. 1.5–2 mm wide. The style and stamens are centrally placed. The anthers may be included in the tube (*I. paniculata*) or, more commonly, are exserted (*I. longituba*, *I. paucifolia*). Perianth color in these tubular flowers is white, cream, or pink, sometimes more darkly pigmented around the throat. All the tubular flowers are unscented.

In the third floral category, typified by *Ixia maculata* (sect. *Ixia*), the perianth forms a broad, flat salver, typically 2–3 cm in diameter, with the tepals extended horizontally at the apex of a filiform floral tube (Fig. 2B, 5). The tube is narrow and completely filled by the style and closed off at the mouth by coherent or coalescent filaments, contains no nectar, and functions as a pedicel, supporting the tepals and stamens. The perianth color is remarkably diverse among species of this group, ranging from white or cream to pink or mauve, or dark red, purple, turquoise, or intense orange or yellow, but flowers are usually brilliantly pigmented. The flowers are unscented and usually have a dark, contrasting central marking, sometimes including the filaments and occasionally the anthers, i.e., beetle marks sensu Goldblatt et al. (1998a). Other authors (Steiner, 1998a, b) have restricted the term beetle mark to describe markings that more closely resemble a beetle, but our broader definition seems useful. The longitudinally dehiscent anthers are centrally placed and the pollen is retained on the dehisced anthers. *Ixia flexuosa* has unusually small flowers for this group, ca. 1.5 mm in diameter, and they are fragrant and borne on particularly slender, wiry peduncles. Its crowded inflorescences tend to droop and wave in the wind, unlike the erect spikes of other species in the group.

The last floral category, represented by *Ixia scillaris* (Fig. 6), is restricted to species of subgenus *Dichone* (Lewis, 1962). The perianth is salver-shaped and pale to deep pink, often darker pink toward the center of the flower. The flowers are secund with tepals vertically oriented. The perianth tube is filiform with the tube wall enveloping the style in a tight, continuous sheath. The tube contains no detectable nectar. The stamens are fully exserted, extending horizontally on short, somewhat stubby filaments. The anthers are 3–4 mm long, usually as long as the filaments, and inflated and basally porose. Dehiscence is delayed and begins from the base and does not reach the anther apices (Fig. 2D). In *I. scillaris* the anthers are more or less unilateral, and lie slightly below horizontal, perhaps due only to gravity. Pollen is retained within anther locules and not exposed as it is in anthers of most other Iridaceae. The anthers have been described as subdidymous (Lewis, 1962), indicating that they have virtually no connective tissue.

We were unable to include *Ixia acaulis* in the study, and the species may represent an additional floral category. Plants are unique in the genus in being acaulescent, and the long-tubed, yellow flowers are borne at ground level with the ovary held below ground (Goldblatt & Manning, 1993). Included in section *Hyalis* by de Vos (1999), *I. acaulis* is unlikely to be pollinated by the long-proboscid flies that visit other species in the section for the flowers are produced in May when no known species of this pollinator group are on the wing.

NECTAR PROFILE

Nectar, when present (Table 2), is produced in septal nectaries in *Ixia* species, as in the entire subfamily Crocoideae (Goldblatt, 1990a, 1991). Nectar is secreted from minute, circular pores at the top of the ovary, flowing directly into the base of the perianth tube, and is typically retained in

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Figures 3–6. Representative species of *Ixia* showing general features of habit and floral form and pigmentation. —3 (top left). *Ixia rapunculoides*, with nodding flowers with a wide tube. —4 (top right). *I. paniculata*, with pale-colored flowers lacking markings, an elongate tube, and the anthers included in the floral tube. —5 (bottom left). *I. curta*, with flowers crowded and with prominent darker, central markings and large anthers. —6 (bottom right). *Ixia scillaris*, with vertically oriented flowers, secund on the spikes, and nodding anthers.

Table 3. Nectar properties of selected *Ixia* flowers. Fru = fructose, Glu = glucose, Suc = sucrose. Nectar sugars analyzed by B.-E. van Wyk. Sample size indicates number of flowers (of different individuals) examined at study site.

Species	Sample size	Nectar volume µl (n)	Nectar % sugar (±SD)	% of different sugars Fru	Glu	Suc	Mean Suc/Glu + Fru (n)
I. longituba	6	2.0–2.4	23.7 (3.3)	14–19	12–22	60–75	1.94 (3)
I. latifolia	5	1.7–2.9	27.6 (2.5)	—	—	—	—
	10	0.4–1.3	38.5 (3.6)	—	—	—	—
I. paucifolia	5	1.1–1.6	29.8 (1.8)	17–21	20–25	54–63	1.41 (2)
I. paniculata	8	3.9–5.7	26.1 (2.7)	16–19	17–23	58–66	1.66 (3)
I. rapunculoides	3	1.1–1.5	28.0 (1.0)	27–28	29–37	35–44	0.69 (3)

(*I. curta, I. flexuosa, I. maculata, I. metelerkampiae, I. monadelpha, I. polystachya, I. scillaris*: tubes filiform, closed at the mouth, and nectar not detectable)

the lower part of the tube. The length of the perianth tube varies among the *Ixia* species (Table 2) examined, ranging from 4 mm in *I. scillaris* to 60 mm in *I. paniculata*. In species with a funnel-shaped tube, the lower, slender part is mostly 4–8 mm long. Nectar is present in flowers of groups 1 and 2 but is not produced in the *Ixia maculata* group or in subgenus *Dichone*. Nectar volumes are modest in the *Ixia latifolia* group, mostly 1.5–3 µl, and rarely exceed 5 µl (Table 3). In the long-tubed *I. paniculata* group nectar secretion ranges from 1.1–1.6 µl (in *I. paucifolia*) to 3.9–5.7 µl (in *I. paniculata*). The nectar is sucrose-rich to sucrose-dominant with sugar solute making up 25–35% of the total volume of fluid (Table 3).

Insect Observation, Pollination Mechanisms, and Pollen Load Analysis

Pollination strategies vary among *Ixia* species and largely correlate with the mode of floral presentation. There appears to be a correlation between pollinators of species in category 1 and the width of the upper part of the perianth tube. Species with a wide, cup-shaped upper tube are pollinated primarily by female *Anthophora diversipes*, a large bee (body 14–17 mm long) with a relatively long proboscis, 6.5–8 mm long (Goldblatt et al., 1998b). These bees land on the flower and brush both the anthers and stigma lobes as they push their heads into the floral cup. *Anthophora diversipes* is a polylectic forager (Table 5), and different individuals were found to carry the pollen of co-blooming Fabaceae, *Salvia* sp. (Lamiaceae), *Lobostemon* (Boraginaceae), and Iridaceae (including *Gladiolus, Hesperantha, Moraea*) in their scopae. One bee, captured on *I. latifolia*, carried three pollinaria of *Satyrium humile* Lindl. (Orchidaceae) on its frons. We have also captured the nemestrinid fly, *Prosoeca sp.* (mouth parts 12 mm long), foraging for nectar on *I. rapunculoides*. The fly grasps the extended tepals with its tarsi while continuing to vibrate its wings.

Among the species of category 1, those with a narrow upper floral tube have a different range of insect visitors. *Ixia orientalis* is visited by the butterfly *Colias electo* (Pieriidae). This insect lands on the tepals and its head contacts anthers and stigma lobes while it extends its proboscis into the floral tube. The small-flowered and short-tubed *Ixia orientalis* has a particularly narrow floral tube, and it appears to be visited only by this insect.

Ixia aurea and *I. tenuifolia* are visited by a combination of short-proboscid tabanid flies, *Mesomyia edentula* or *Philoliche atricornis* (proboscis 3–5 mm long), respectively, and hopliine beetles (Scarabaeidae) (Table 5), while *P. atricornis* alone was captured on *I. esterhuyseniae*. The tabanid flies foraged for nectar on, and carried mixed loads of pollen of, *Ixia, Ornithogalum thyrsiflora* Jacq. (Hyacinthaceae), and Asteraceae. Beetles ignored the nectar in the floral tube, feeding exclusively on pollen, and contacted both dehisced anthers and stigma lobes while foraging, copulating, or engaging in agonistic behavior (see Goldblatt et al., 1998a). These beetles also carried mixed loads of *Ixia, Ornithogalum,* and asteraceous pollen. Both these short-proboscid tabanid flies and the hopliine beetles carried pollen in the dorsal and ventral parts of their bodies as well as the frons. Pollen of *Ixia* species was, however, located on the frons and dorsal part of the thorax in the tabanids, reflecting their body orientation as they foraged for nectar on *Ixia* flowers.

Tubular flowers (category 2) were visited by long-proboscid flies, either Nemestrinidae or Tabanidae, with probosces between 18 and 70 mm long (Table

Table 4. Comparison of the length of perianth tube and mouth parts of long-proboscid flies collected on *Ixia* species. Measurements are from study sites and represent the ranges found by sampling flowers of 10 individuals. For insects only three individuals were captured and measured.

Plant species	Perianth tube length (mm)	Proboscis length (mm)	Insect species
I. paniculata	65–75	67–70	*Moegistorhynchus longirostris*
I. longituba	30–32	22–24	*Philoliche gulosa*
I. paucifolia	16–28	18–22	*P. gulosa*

4). These flies grasp the tepals with their tarsi and probe for nectar while continuing to vibrate their wings. Field observations and pollen load analyses show that long-proboscid flies visit *Ixia* species during the same foraging bouts in which they visit open flowers of *Pelargonium* species (Geraniaceae) and species of several genera of Iridaceae (including *Babiana, Gladiolus, Geissorhiza,* and *Lapeirousia* species). One specimen of *Moegistorhynchus* also carried one pollinarium of *Disa draconis* (L. f.) Sw. (Orchidaceae), also a member of the guild of plant species that depend on this particular fly for pollen dispersal (Manning & Goldblatt, 1997; Johnson & Steiner, 1997).

Flowers of category 3, section *Ixia* (with a salver-shaped perianth and longitudinally dehiscent anthers), are visited primarily by hopliine beetles (Table 5), but one species is visited primarily by honey bees (*Apis mellifera*). Observation and pollen load analyses of beetles captured on species of the *Ixia maculata* group show unusually diverse foraging behavior, visiting flowers of a range of species including other Iridaceae (e.g., *Gladiolus, Moraea, Romulea*), Asteraceae, *Monsonia* (Geraniaceae), *Prismatocarpus pedunculatus* (P. J. Bergius) A. DC. (Campanulaceae), *Spiloxene capensis* (L.) Garside (Hypoxidaceae), and *Ornithogalum* spp. When *Apis mellifera* visited *I. flexuosa* it contacted the stigmatic lobes while scraping anthers for pollen. Captured individuals of *Apis mellifera* carried pure loads of *Ixia* pollen on their bodies and in their corbiculae (Table 5).

At one study site, Bainskloof, we noted a possible example of Batesian mimicry. *Ixia metelerkampiae,* which has pale mauve flowers with a darker central area, and co-blooming *Thereianthus ixioides* G. J. Lewis (also Iridaceae), with whitish flowers also with purple markings in the center, closely re-

Table 5. Pollen load analysis of captured insects on *Ixia* species. Scarabaeidae: *Anisochelus, Chasme, Heterochelus, Lepisia, Lepithrix, Pachycnema, Peritrichia, Scelophysa.* Apoidea: Apidae: *Amegilla, Anthophora, Apis, Pachymelus.* Melittidae: *Rediviva.* Nemestrinidae: *Moegistrohynchus, Prosoeca.* Tabanidae: *Mesomyia, Philoliche.* In addition to the insects listed below, two individuals of *Rediviva aurata,* and one each of *Andrena* sp. and *Colletes* sp. captured on *I. scillaris* carried pollen only of non-host species, and one *Lepithrix* individual captured on *I. tenuifolia* carried no pollen at all.

Plant and [insect] taxon	Number of insects carrying pollen load(s): Host flr only	Host flr plus other sp.
Subgenus *Ixia*: Section *Morphixia*		
I. aurea		
[*Heterochelus arthriticus*]	3	2
[*Lepithrix ornatella*]	0	1
[*Pachycnema crassipes*]	0	2
[*Mesomyia edentula*]	2	2
I. capillaris		
[*Anthophora diversipes* ♀]	0	2
I. esterhuyseniae		
[*Philoliche atricornis*]	0	1
I. latifolia 1		
[*Anthophora diversipes* ♀] popln 2	0	2
[*Anthophora diversipes* ♀]	0	2
[*A. schulzei* ♀]	0	2
[*Amegilla spilostoma* ♀]	0	1
I. odorata		
[*Anthophora diversipes* ♀]	0	2
I. orientalis		
[*Colias electo*]	3	0
I. rapunculoides		
[*Anthophora schulzei* ♀]	0	1
[*Pachymelus peringueyi* ♀]	0	2
I. tenuifolia		
[*Lepithrix ornatella*]	0	5
[*Philoliche atricornis*]	3	3
I. thomasiae		
[*Anthophora diversipes* ♀]	0	2
Section *Hyalis*		
I. longtuba		
[*Philoliche gulosa*]	0	2
I. paniculata		
[*Moegistorhynchus longirostris*]	0	2
I. paucifolia (site 1)		
[*Philoliche gulosa*] (site 2)	0	3
[*Philoliche gulosa*]	0	2

Table 5. Continued.

Plant and [insect] taxon	Number of insects carrying pollen load(s): Host flr only	Host flr plus other sp.
Section *Ixia*		
I. atrandra		
[*Peritrichia subsquamosa*]	0	3
I. curta		
[*Lepisia rupicola*]	0	3
[*Lepithrix fulvipes*]	0	5
[*Pachycnema crassipes*]	0	1
I. flexuosa		
[*Apix mellifera*]	3	0
I. lutea (site 1)		
[*Lepithrix ornatella*]	0	5
(site 2)		
[*Anisochelus inornatus*]	1	4
(site 3)		
[*Anisochelus inornatus*]	1	3
[*Chasme decora*]	0	1
[*Peritrichia rufotibialis*]	1	1
I. maculata		
(site 1)		
[*Pachynema crassipes*]	0	2
(site 2)		
[*Pachycnema crassipes*]	0	2
[*Lepithrix fulvipes*]	0	3
(site 3)		
[*Heterochelis sexlineatus*]	0	1
[*Scelophysa militaris*]	0	1
[*Pachycnema crassipes*]	0	1
[*Lepithrix ornatella*]	0	5
I. metelerkampiae		
[*Peritrichia* sp.]	1	2
I. monadelpha		
[*Lepithrix fulvipes*]	0	1
I. versicolor		
[*Peritrichia pseudursa*]	0	4
Subgenus *Dichone*		
I. scillaris (site 1)		
[*Amegilla spilostoma* ♀]	0	4
(site 2)		
[*Rediviva aurata* ♀]	0	0
[*Colletes* sp. ♂]	0	0
[*Andrena* sp. ♀]	0	0
Totals	18	93

semble one another as regards floral presentation, and they appear to be visited indiscriminately by the same beetle, *Peritrichia* sp. Both species have a filiform floral tube in which the style is tightly enclosed by the walls of the tube. In *T. ixioides* small amounts of nectar are produced and are visible at the mouth of the tube. This suggests that the species may offer a secondary reward and may have a more generalist pollination system than *I. metelerkampiae*, which does not offer nectar.

Category 4 flowers of subgenus *Dichone* (with secund, salver-shaped flowers and basally dehiscent anthers) appear to be pollinated by female *Amegilla* (Apidae). These bees have bodies 10–12 mm long, thus smaller than *Anthophora diversipes*, although they also have an elongated proboscis (Goldblatt et al., 1998b). These bees cling to the anthers of *Ixia scillaris* while foraging exclusively for pollen. Pollen removal is accomplished by thoracic vibration in which the foraging female produces the characteristic high-pitched whine (sensu Buchmann, 1983). A bee did not remain on an individual flower of *I. scillaris* for longer than 1.5 seconds. The head of a bee appeared to contact one of the three extended stylar lobes while it grasped the anthers. Pollen load analyses of *Amegilla* captured on *I. scillaris* (Table 5) showed that the bees also foraged on nectariferous flowers of *Aspalathus* (Fabaceae) and *Muraltia* (Polygalaceae). An interesting aspect of the pollination of *I. scillaris* is that although the anthers are prominently displayed and evidently attractive to bees other than *Amegilla*, the melittid, *Rediviva aurata*, as well as *Andrena* sp. and *Colletes* sp. seen visiting flowers and clasping the anthers, did not carry pollen of the species. This is evidently a reflection of these bees' inability to vibrate their wings at the necessary frequency to dislodge pollen from the anthers.

Discussion

The adaptive radiation of pollination mechanisms in *Ixia* is consistent with aspects of pollination systems described in *Lapeirousia* (Goldblatt et al., 1995), *Moraea* (Goldblatt et al., 1989; Goldblatt & Bernhardt, 1999), and *Gladiolus* (Goldblatt & Manning, 1998; Goldblatt et al., 1998b). Specifically, pollination systems in moderate to large-sized genera of Iridaceae in Africa diverge as different species in the same genus exploit different pollen vectors. *Lapeirousia* (Goldblatt, 1990b; Goldblatt & Manning, 1992) has specialized species that utilize long-proboscid Tabanidae, Nemestrinidae, or sphinx moths, as well as species with generalist systems that include various long-

tongued bees, bee flies (Bombyliidae), and butterflies. Pollination in the much larger genus *Gladiolus*, with some 245 sub-Saharan African species, is dominated by long-tongued anthophorine bees, but pollination by long-proboscid Nemestrinidae and Tabanidae, the large butterfly, *Aeropetes tulbaghia*, night-flying moths, and sunbirds (*Nectarinia* spp.) has also been observed in several species. *Moraea* species appear to be pollinated primarily by bees with elongated or extended mouth parts (Goldblatt et al., 1989). However, recent work by Steiner (1998a) and Goldblatt et al. (1998a) shows that several southwestern Cape species are pollinated by hopliine beetles, while other species are pollinated only by pollen-collecting bees, or a combination of bees, muscid and scathophagid flies, and hopliine beetles (Goldblatt & Bernhardt, 1999). Like these other genera of the Iridaceae, *Ixia* also has species adapted to a range of pollination systems. The flowers of *Ixia* species thus show different sets of correlated morphological specializations that represent co-adaptations with different floral foragers.

Pollination by hopliine beetles in *Ixia* parallels pollination by hopliine beetles in other genera of the Iridaceae. As in *Moraea insolens* Goldblatt, *M.* cf. *lurida* Ker-Gawl., *M. tulbaghensis* L. Bolus, and *M. villosa* (Ker-Gawl.) Ker-Gawl. (Goldblatt et al., 1998a; Steiner, 1998a), the flowers of the *Ixia maculata* group have darkened floral markings and large areas of flat surface, brightly colored pollen, and little or no nectar. Pollination by nectar-foraging anthophorine bees in some species of *Ixia* parallels that in *Moraea inclinata*, many *Gladiolus* species, and *Lapeirousia divaricata* N. E. Br. (Goldblatt et al., 1989, 1995, 1998b). These *Ixia* species also have flowers broad enough to offer a landing platform, pigmentation patterns mostly in the pink to blue range, inconspicuous stamens, and nectar retained at the base of a floral tube.

The adaptive radiation of pollination mechanisms in *Ixia*, however, appears less diverse than in *Lapeirousia* or *Gladiolus*. Neither moth nor bird pollination appears to occur in the genus. While butterfly pollination is described here in *Ixia*, *I. orientalis* is definitely not part of the southern African red-flowered, summer-blooming *Aeropetes* butterfly guild described by Johnson and Bond (1994).

The unique feature of pollination systems in *Ixia* is represented by the unusual buzz-pollination system in subgenus *Dichone*. In other genera of Iridaceae in southern Africa there is a divergence between bee pollination in nectarless versus nectariferous flowers, correlated with either pollen or nectar as the reward. For example, most species of *Gladiolus* are nectariferous, and large anthophorines forage on gullet- or flag-shaped flowers (sensu Faegri & van der Pijl, 1979). *Gladiolus quadrangulus* (D. Delaroche) Barnard and *G. stellatus* G. J. Lewis have stellate flowers, and *Andrena* sp. or *Apis mellifera* scrape pollen from prominent, exserted anthers (Goldblatt et al., 1998b). A similar divergence is also found in bee-pollinated species of *Moraea* (Goldblatt & Bernhardt, 1999).

The genus *Ixia* actually exhibits three different modes of bee pollination. The nectar-bearing tube and cup-shaped floral system of section *Morphixia* and the pollen-rich, nectarless, salver-shaped floral system of *I. flexuosa* parallel systems described in *Gladiolus* (Goldblatt et al., 1998b). However, buzz pollination described in subgenus *Dichone* has not been observed in any other genus of the Iridaceae to date. Harris (1905) noted that porose or porate anthers are found in the Iridaceae, but Buchmann (1983) did not mention buzz-pollinated flowers in the family in his review of the subject. Buchmann showed how varied pore position may be on a solitary anther but did not mention basal pores or basal dehiscence. Buzz pollination in subgenus *Dichone* has evolved independently, adding another example to the widespread convergence within the monocots for buzz pollination. Buzz pollination has been reported within the petaloid monocots in *Cyanella* (Tecophilaeaceae—Dulberger & Ornduff, 1980), *Echeandia* (Anthericaceae—Bernhardt & Montalvo, 1979), *Dianella* (Hemerocallidaceae—Bernhardt, 1995), *Dichopogon* (Lomandraceae—Bernhardt & Burns-Balogh, 1986; see Bernhardt, 1996, fig. 3), and *Xiphidium* (Haemodoraceae—Buchmann, 1980). In these taxa filaments are relatively short and anthers are inflated. Buzz-pollinated species of *Ixia*, however, have one unusual character found only in a few buzz-pollinated angiosperms such as the Australian *Calectesia* (Dasypogonaceae) and *Hibbertia* (Dilleniaceae). In these last two taxa the stylar lobes diverge, forming a triangular perimeter outside the centrally placed anthers (Bernhardt, 1986). We presume that this alignment of sexual organs may encourage cross pollination, as a bee should contact stigmatic surfaces before it vibrates the anthers.

Flowers of *Ixia flexuosa* appear to represent a secondary shift to bee pollination within the predominantly beetle-pollinated section *Ixia*. The flowers have the morphological modifications of hopliine beetle-pollinated species of the section, but the flowers are relatively small, ca. 15 mm in diameter, too small to easily accommodate more than one beetle at a time. Moreover, they are scented and also often fairly dull-colored, either cream or mauve

(but sometimes pink) and without a prominent central mark, features not normally associated with hopliine pollination (Picker & Midgley, 1996; Steiner, 1998b; Goldblatt et al., 1998b). The entire inflorescence often droops and waves about in the wind on a slender peduncle, making the flowers poor sites for beetle assembly. Instead, the flowers seem to offer pollen to foraging bees. *Apis mellifera* is a common visitor, which we have seen actively collecting pollen on the species. The corbiculae of bees captured on *I. flexuosa* contained pure loads of *Ixia* pollen.

Outgroup comparison with the genera most closely allied to *Ixia, Sparaxis,* and *Tritonia* (Lewis, 1962; Goldblatt et al., 2000), suggests that the ancestral pollination system in *Ixia* is the one in which flowers are cup-shaped, produce nectar, and are pollinated by large anthophorine bees. This is the plesiomorphic pollination strategy in *Sparaxis* and *Tritonia* according to current views on the evolution of these genera. In both genera a zygomorphic, bilabiate flower that produces nectar is believed to be ancestral, as it probably is for the entire subfamily Ixioiodeae (Goldblatt, 2000). All *Ixia* species have a radially symmetric perianth, a major synapomorphy for the genus. Flowers of species of sections *Morphixia* and *Hyalis* still produce nectar in a hollow perianth tube. Specialization for pollinators in *Ixia* has shifted in two major directions.

An emphasis on nectar as the sole reward accompanied by an elongation of the floral tube has occurred in species of section *Hyalis* and is the only system known to be utilized by any species of the section. This pollination system is most highly developed in *Ixia paniculata,* which has a floral tube up to 65(–75) mm long and is the only species of the section visited by *Moegistorhynchus longirostris.* Other members of the section utilize shorter-proboscid Tabanidae. In neither fly family is pollen consumed, and anthers of *I. paniculata* are actually concealed within the floral tube, a mode of presentation also found in *I. fucata* Ker-Gawl. (Lewis, 1962), on which we have not captured any insects.

A second direction of morphological evolution has been the reduction of the perianth tube in width and sometimes length, accompanied by the suppression of nectar production. In most species of section *Ixia* ample pollen is produced by longitudinally dehiscent anthers, but the flowers primarily appear to constitute sites for the assembly and mating of species of hopliine beetles. While these beetles do consume pollen, they spend most of their time on flowers either crawling over the perianth or lying with their heads in the center of a flower. Beetle pollination is a strategy adopted by many southern African petaloid geophytes, as well as annual and perennial Asteraceae, and is known in the Iridaceae in *Aristea, Moraea, Hesperantha vaginata* (Sweet) Goldblatt, and *Romulea* (Goldblatt & Manning, 1996; Steiner, 1998a; Goldblatt et al., 1998a, 2000). Pollination by hopliine beetles also occurs in other genera, either alone or in combination with other insects, usually nectarivorous, short-proboscid Tabanidae (e.g., species of *Sparaxis*) or with pollen-collecting female halictid and andrenid bees and *Apis mellifera* (e.g., species of *Romulea, Gladiolus meliusculus* (G. J. Lewis) Goldblatt & J. C. Manning). Hopliine beetle pollination, however, appears to be particularly well developed in *Ixia* sect. *Ixia,* a taxon that appears to comprise a clade, defined by the morphological adaptations associated with the pollination system. These include the reduction in the diameter of the perianth tube, which is filiform and tightly sheaths the style, and the closure of the apex of the tube by broad-based filaments, inserted at the base of the tepals and often united below or entirely. Nectar production is also suppressed, and the floral tube, a plesiomorphic structure in Iridaceae subfamily Crocoideae, simply functions as a stalk for the petaloid, salver-shaped part of the flowers. Additional adaptations include the development of bright floral pigmentation, mostly orange, yellow, red, or purple, often with dark centers that appear to represent beetle marks, a common feature of beetle flowers (Picker & Midgley, 1996). *Ixia aurea* and *I. tenuifolia* (sect. *Morphixia*) offer a remarkable example within the genus of convergence for hopliine beetle pollination. Both species have a narrowly funnel-shaped floral tube that contains nectar, but the floral pigmentation is like that of species of section *Ixia,* being deep yellow to orange, and with a dark center in *I. tenuifolia.* They are pollinated by a combination of hopliine beetles and the tabanid flies *Mesomyia edentula* and *Philoliche atricornis.* This system offers a striking parallel with the beetle pollination system in *Sparaxis* species (Goldblatt et al., 1998b, 2000) in which small amounts of nectar are also offered.

While there are far fewer species in *Ixia* compared to *Gladiolus* and *Moraea,* pollination systems of *Ixia* are relatively diverse. The diversity of pollination systems in *Ixia* reflects relatively minor changes in floral characters based on changes in pigmentation, the presence or absence of nectar, floral tube, and mode of anther dehiscence. With the exception of the mode of anther dehiscence, all other floral modifications in *Ixia* parallel those found in allied genera of the family.

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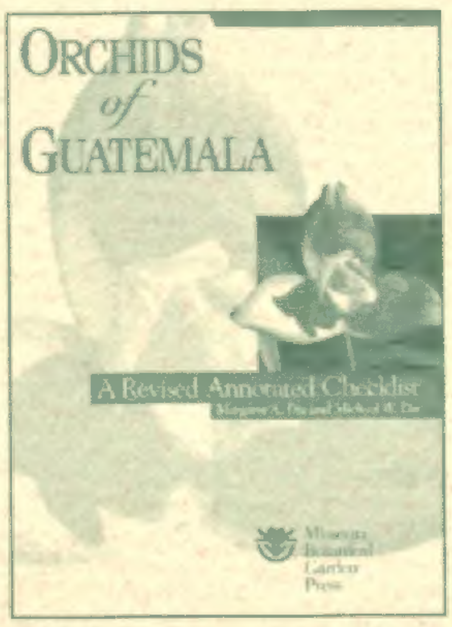

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